

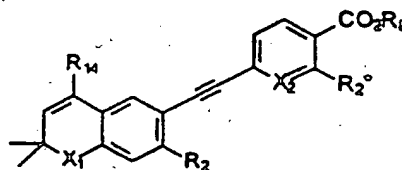
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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/US98/27314  (22) International Filing Date: 21 December 1998 (21.12.98)  (30) Priority Data: 08/998,319 24 December 1997 (24.12.97) US  (71) Applicant: ALLERGAN SALES, INC. [US/US]; 2525 Dupont Drive, Irvine, CA 92612 (US).  (72) Inventors: KLEIN, Elliott, S.; 13211-C Admiral Avenue, Marina del Rey, CA 90292 (US). JOHNSON, Alan, T.; 7 Via Arribo, Rancho Santa Margarita, CA 92688 (US). STANDEVEN, Andrew, M.; 427 1/2 Orchid Avenue, Corona del Mar, CA 92625 (US). BEARD, Richard, L.; 2341 Azure Avenue, Newport Beach, CA 92660 (US). GILLET, Samuel, J.; 545 Pierce Street #2407, Albany, CA 94706 (US). DUONG, Tien, T.; 267 Orange Blossom, Irvine, CA 92620 (US). NAGPAL, Sunil; 22031 Elsberry, Lake Forest, CA 92630 (US). VULIGONDA, Vidyasagar, 15 Sweet Rain, Irvine, CA 92614 (US). TENG, Min; 5185 Seachase Street, San Diego, CA 92130 (US). CHANDRARATNA, Roshantha, A.; 25841 Empresa, Mission Viejo, CA 92691 (US).		(74) Agents: BARAN, Robert, J. et al.; Allergan Sales, Inc., 2525 Dupont Drive, Irvine, CA 92612 (US).  (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.
(54) Title: BENZOPYRAN AND BENZOTHIOPYRAN DERIVATIVES HAVING RETINOID ANTAGONIST-LIKE ACTIVITY  (57) Abstract  2,2-Dialkyl- 4-aryl-substituted benzopyran and benzothiopyran derivatives of formula (I) where the symbols have the meaning described in the specification, have retinoid negative hormone and/or antagonist-like biological activities. The invented RAR antagonists can be administered to mammals, including humans, for the purpose of preventing or diminishing action or RAR agonists on the bound receptor sites. Specifically, the RAR agonists are administered or coadministered with retinoid drugs to prevent or ameliorate toxicity or side effects caused by retinoids or vitamin A or vitamin A precursors. The retinoid negative hormones can be used to potentiate the activities of other retinoids and nuclear receptor agonists.		



(I)

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BENZOPYRAN AND BENZOTHIOPYRAN DERIVATIVES HAVING RETINOID  
ANTAGONIST-LIKE ACTIVITY

Related Applications

This application is a continuation-in-part of application serial number 08\871,093 filed on June 9, 1997, which is a divisional of application serial number 08\613,863 filed on March 11, 1996 which in turn claims the benefit of priority under 35 U.S.C. § 119(e) of the three following U.S. applications, each of which was filed as a nonprovisional application and converted to a provisional application by separate petitions filed on January 31, 1996: Application No. 08/522,778, filed September 1, 1995; now provisional application serial number 60/019,015; Application No. 08/522,779, filed September 1, 1995, now provisional application serial number \_\_\_\_\_; and Application No. 08/542,648, filed October 13, 1995, now provisional application serial number 60/020,501. The complete disclosures of these related applications are hereby incorporated herein by this reference thereto.

Field of the Invention

The present invention relates to novel compounds having retinoid negative hormone and/or retinoid antagonist-like biological activities. More specifically, the invention relates to 4-aryl substituted benzopyran, 4-aryl substituted benzothiopyran, 4-aryl substituted 1,2-dihydroquinoline and 8-aryl substituted 5,6-dihydronaphthalene derivatives which may also be substituted by a substituted 3-oxo-1-propenyl group. These novel compounds have retinoid antagonist like-activity and are useful for treating or preventing retinoid and vitamin A and vitamin A precursor induced toxicity in mammals and as an adjunct to treatment of mammals with retinoids to prevent or ameliorate unwanted or undesired side effects. The invention further relates to the use of retinoid negative hormones for increasing the biological activities of

1 other retinoids and steroid hormones and inhibiting the basal activity of  
2 unliganded retinoic acid receptors.

3 Background of the Invention

4 Compounds which have retinoid-like activity are well known in  
5 the art, and are described in numerous United States and other patents  
6 and in scientific publications. It is generally known and accepted in the  
7 art that retinoid-like activity is useful for treating mammals, including  
8 humans, in order to cure or alleviate the symptoms associated with  
9 numerous diseases and conditions.

10 Retinoids (vitamin A and its derivatives) are known to have broad  
11 activities, including effects on cell proliferation and differentiation, in a  
12 variety of biological systems. This activity has made retinoids useful in  
13 the treatment of a variety of diseases, including dermatological disorders  
14 and cancers. The prior art has developed a large number of chemical  
15 compounds which have retinoid-like biological activity, and voluminous  
16 patent and chemical literature exists describing such compounds. The  
17 relevant patent literature includes United States Patent Nos. 4,980,369,  
18 5,006,550, 5,015,658, 5,045,551, 5,089,509, 5,134,159, 5,162,546,  
19 5,234,926, 5,248,777, 5,264,578, 5,272,156, 5,278,318, 5,324,744,  
20 5,346,895, 5,346,915, 5,348,972, 5,348,975, 5,380,877, 5,399,561,  
21 5,407,937, (assigned to the same assignee as the present application) and  
22 patents and publications cited therein, which particularly describe or  
23 relate to chroman, thiochroman and 1,2,3,4-tetrahydroquinoline  
24 derivatives which have retinoid-like biological activity. In addition,  
25 several applications are pending which are assigned to the assignee of  
26 the present application, and which are directed to further compounds  
27 having retinoid-like activity.

28 United States Patent Nos. 4,740,519 (Shroot et al.), 4,826,969  
29 (Maignan et al.), 4,326,055 (Loeliger et al.), 5,130,335 (Chandraratna et



1 al.), 5,037,825 (Klaus et al.), 5,231,113 (Chandraratna et al.), 5,324,840  
2 (Chandraratna), 5,344,959 (Chandraratna), 5,130,335 (Chandraratna et  
3 al.), Published European Patent Application Nos. 0 176 034 A (Wuest  
4 et al.), 0 350 846 A (Klaus et al.), 0 176 032 A (Frickel et al.), 0 176 033  
5 A (Frickel et al.), 0 253 302 A (Klaus et al.), 0 303 915 A (Bryce et al.),  
6 UK Patent Application GB 2190378 A (Klaus et al.), German Patent  
7 Application Nos. DE 3715955 A1 (Klaus et al.), DE 3602473 A1 (Wuest  
8 et al., and the articles *J. Amer. Acad. Derm.* 15: 756 - 764 (1986) (Sporn  
9 et al.), *Chem. Pharm. Bull.* 33: 404-407 (1985) (Shudo et al.), *J. Med*  
10 *Chem.* 31: 2182 - 2192 (1988) (Kagechika et al.), Chemistry and Biology  
11 of Synthetic Retinoids CRC Press Inc. 1990 pp. 334 - 335, 354 (Dawson  
12 et al.), describe or relate to compounds which include a  
13 tetrahydronaphthyl moiety and have retinoid-like or related biological  
14 activity. United States Patent No. 4,391,731 (Boller et al.) describes  
15 tetrahydronaphthalene derivatives which are useful in liquid crystal  
16 compositions.

17 An article by Kagechika et al. in *J. Med. Chem* 32:834 (1989)  
18 describe certain 6-(3-oxo-1-propenyl)- 1,2,3,4-tetramethyl- 1,2,3,4-  
19 tetrahydronaphthalene derivatives and related flavone compounds  
20 having retinoid-like activity. The articles by Shudo et al. in *Chem.*  
21 *Pharm. Bull.* 33:404 (1985) and by Jetten et al. in *Cancer Research*  
22 47:3523 (1987) describe or relate to further 3-oxo-1-propenyl derivatives  
23 (chalcone compounds) and their retinoid-like or related biological  
24 activity.

25 Unfortunately, compounds having retinoid-like activity (retinoids)  
26 also cause a number of undesired side effects at therapeutic dose levels,  
27 including headache, teratogenesis, mucocutaneous toxicity,  
28 musculoskeletal toxicity, dyslipidemias, skin irritation, headache and  
29 hepatotoxicity. These side effects limit the acceptability and utility of

1 retinoids for treating disease.

2 It is now general knowledge in the art that two main types of  
3 retinoid receptors exist in mammals (and other organisms). The two  
4 main types or families of receptors are respectively designated as the  
5 RARs and RXRs. Within each type there are subtypes: in the RAR  
6 family the subtypes are designated RAR- $\alpha$ , RAR- $\beta$  and RAR- $\gamma$ , in  
7 RXR the subtypes are: RXR- $\alpha$ , RXB- $\beta$  and RXR- $\gamma$ . Both families of  
8 receptors are transcription factors that can be distinguished from each  
9 other based on their ligand binding specificities. All-*trans*-RA (ATRA)  
10 binds and activates a class of retinoic acid receptors (RARs) that  
11 includes RAR- $\alpha$ , RAR- $\beta$  and RAR- $\gamma$ . A different ligand, 9-*cis*-RA (9C-  
12 RA), binds and activates both the RARs and members of the retinoid X  
13 receptor (RXR) family.

14 It has also been established in the art that the distribution of the  
15 two main retinoid receptor types, and of the several subtypes is not  
16 uniform in the various tissues and organs of mammalian organisms.  
17 Moreover, it is generally accepted in the art that many unwanted side  
18 effects of retinoids are mediated by one or more of the RAR receptor  
19 subtypes. Accordingly, among compounds having agonist-like activity at  
20 retinoid receptors, specificity or selectivity for one of the main types or  
21 families, and even specificity or selectivity for one or more subtypes  
22 within a family of receptors, is considered a desirable pharmacological  
23 property.

24 Relatively recently compounds have been developed in the art  
25 which bind to RAR receptors without triggering the response or  
26 responses that are triggered by agonists of the same receptors. The  
27 compounds or agents which bind to RAR receptors without triggering a  
28 "retinoid" response are thus capable of blocking (to lesser or greater  
29 extent) the activity of RAR agonists in biological assays and systems.

1 More particularly, regarding the scientific and patent literature in this  
2 field, published PCT Application WO 94/14777 describes certain  
3 heterocyclic carboxylic acid derivatives which bind to RAR retinoid  
4 receptors and are said in the application to be useful for treatment of  
5 certain diseases or conditions, such as acne, psoriasis, rheumatoid  
6 arthritis and viral infections. A similar disclosure is made in the article  
7 by Yoshimura et al. *J Med. Chem.* 38: 3163-3173 (1995). Kaneko et al.  
8 *Med. Chem Res.* 1:220-225 (1991); Apfel et al. *Proc. Natl. Acad. Sci.*  
9 *USA* 89: 7129-7133 Augusty 1992 *Cell Biology*; Eckhardt et al. *Toxicology*  
10 *Letters* 70:299-308 (1994); Keidel et al. *Molecular and Cellular Biology*  
11 14:287-298 (1994); and Eyrolles et al. *J. Med. Chem.* 37: 1508-1517  
12 (1994) describe compounds which have antagonist like activity at one or  
13 more of the RAR retinoid subtypes.

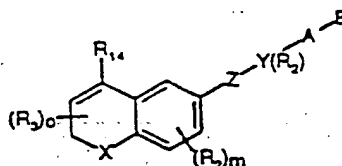
14 In addition to undesirable side-effects of therapy with retinoid  
15 compounds, there occurs occasionally a serious medical condition  
16 caused by vitamin A or vitamin A precursor overdose, resulting either  
17 from the excessive intake of vitamin supplements or the ingestion of  
18 liver of certain fish and animals that contain high levels of the vitamin.  
19 The chronic or acute toxicities observed with hypervitaminosis A  
20 syndrome include headache, skin peeling, bone toxicity, dyslipidemias,  
21 etc. In recent years, it has become apparent that the toxicities observed  
22 with vitamin A analogs, i.e., retinoids, essentially recapitulate those of  
23 hypervitaminosis A syndrome, suggesting a common biological cause,  
24 i.e., RAR activation. These toxicities are presently treated mainly by  
25 supportive measures and by abstaining from further exposure to the  
26 causative agent, whether it be liver, vitamin supplements, or retinoids.  
27 While some of the toxicities resolve with time, others (e.g., premature  
28 epiphyseal plate closure) are permanent.

29 Generally speaking, specific antidotes are the best treatment for

1 poisoning by pharmacological agents, but only about two dozen  
 2 chemicals or classes of chemicals out of thousands in existence have  
 3 specific known antidotes. A specific antidote would clearly be of value  
 4 in the treatment of hypervitaminosis A and retinoid toxicity. Indeed, as  
 5 increasingly potent retinoids are used clinically, a specific antidote for  
 6 retinoid poisoning could be life saving.

### 7 Summary of the Invention

8 The present invention covers compounds of Formula 1



### 15 Formula 1

16 wherein X is S, O, NR' where R' is H or alkyl of 1 to 6 carbons,

17 or

18 X is  $[C(R_1)_2]_n$  where  $R_1$  is independently H or alkyl of 1 to 6  
 19 carbons, and n is an integer between 0 and 2;

20  $R_2$  is hydrogen, lower alkyl of 1 to 6 carbons, F, Cl, Br, I,  $CF_3$ ,  
 21 fluoro substituted alkyl of 1 to 6 carbons, OH, SH, alkoxy of 1 to 6  
 22 carbons, or alkylthio of 1 to 6 carbons;

23  $R_3$  is hydrogen, lower alkyl of 1 to 6 carbons or F;

24 m is an integer having the value of 0 - 3;

25 o is an integer having the value of 0 - 3;

26 Z is  $-C\equiv C-$ ,

27  $-N=N-$ ,

28  $-N=CR_1-$ ,

29  $-CR_1=N$ ,

1  $-(CR_1=CR_1)_{n'}$  where  $n'$  is an integer having the value 0 - 5,  
 2  $-CO-NR_1-$ ,  
 3  $-CS-NR_1-$ ,  
 4  $-NR_1-CO$ ,  
 5  $-NR_1-CS$ ,  
 6  $-COO-$ ,  
 7  $-OCO-$ ,  
 8  $-CSO-$ ,  
 9  $-OCS-$ ;

10 Y is a phenyl or naphthyl group, or heteroaryl selected from a  
 11 group consisting of pyridyl, thienyl, furyl, pyridazinyl, pyrimidinyl,  
 12 pyrazinyl, thiazolyl, oxazolyl, imidazolyl and pyrazolyl, said phenyl and  
 13 heteroaryl groups being optionally substituted with one or two  $R_2$   
 14 groups, or

15 when Z is  $-(CR_1=CR_1)_{n'}$  and  $n'$  is 3, 4 or 5 then Y represents a  
 16 direct valence bond between said  $(CR_2=CR_2)_{n'}$  group and B;

17 A is  $(CH_2)_q$  where q is 0-5, lower branched chain alkyl having 3-6  
 18 carbons, cycloalkyl having 3-6 carbons, alkenyl having 2-6 carbons and 1  
 19 or 2 double bonds, alkynyl having 2-6 carbons and 1 or 2 triple bonds;

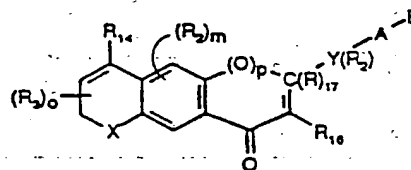
20 B is hydrogen, COOH or a pharmaceutically acceptable salt  
 21 thereof,  $COOR_8$ ,  $CONR_9R_{10}$ ,  $-CH_2OH$ ,  $CH_2OR_{11}$ ,  $CH_2OCOR_{11}$ ,  $CHO$ ,  
 22  $CH(OR_{12})_2$ ,  $CHOR_{13}O$ ,  $-COR_7$ ,  $CR_7(OR_{12})_2$ ,  $CR_7OR_{13}O$ , or tri-lower  
 23 alkylsilyl, where  $R_7$  is an alkyl, cycloalkyl or alkenyl group containing 1  
 24 to 5 carbons,  $R_8$  is an alkyl group of 1 to 10 carbons or  
 25 trimethylsilylalkyl where the alkyl group has 1 to 10 carbons, or a  
 26 cycloalkyl group of 5 to 10 carbons, or  $R_8$  is phenyl or lower  
 27 alkylphenyl,  $R_9$  and  $R_{10}$  independently are hydrogen, an alkyl group of 1  
 28 to 10 carbons, or a cycloalkyl group of 5-10 carbons, or phenyl or lower  
 29 alkylphenyl,  $R_{11}$  is lower alkyl, phenyl or lower alkylphenyl,  $R_{12}$  is lower

1 alkyl, and  $R_{13}$  is divalent alkyl radical of 2-5 carbons, and

2  $R_{14}$  is  $(R_{15})_r$ -phenyl,  $(R_{15})_r$ -naphthyl, or  $(R_{15})_r$ -heteroaryl where  
3 the heteroaryl group has 1 to 3 heteroatoms selected from the group  
4 consisting of O, S and N,  $r$  is an integer having the values of 0 - 5, and

5  $R_{15}$  is independently H, F, Cl, Br, I,  $NO_2$ ,  $N(R_8)_2$ ,  $N(R_8)COR_8$ ,  
6  $NR_8CON(R_8)_2$ , OH,  $OCOR_8$ ,  $OR_8$ , CN, an alkyl group having 1 to 10  
7 carbons, fluoro substituted alkyl group having 1 to 10 carbons, an  
8 alkenyl group having 1 to 10 carbons and 1 to 3 double bonds, alkynyl  
9 group having 1 to 10 carbons and 1 to 3 triple bonds, or a trialkylsilyl or  
10 trialkylsilyloxy group where the alkyl groups independently have 1 to 6  
11 carbons.

12 The present invention further covers compounds of Formula 101



19 Formula 101

20 wherein X is S, O,  $NR'$  where  $R'$  is H or alkyl of 1 to 6 carbons,

21 or

22 X is  $[C(R_1)_2]_n$  where  $R_1$  is independently H or alkyl of 1 to 6  
23 carbons, and  $n$  is an integer between 0 and 2;

24  $R_2$  is hydrogen, lower alkyl of 1 to 6 carbons, F, Cl, Br, I,  $CF_3$ ,  
25 fluoro substituted alkyl of 1 to 6 carbons, OH, SH, alkoxy of 1 to 6  
26 carbons, or alkylthio of 1 to 6 carbons;

27  $R_3$  is hydrogen, lower alkyl of 1 to 6 carbons or F;

28  $m$  is an integer having the value of 0 - 3;

29  $o$  is an integer having the value of 0 - 3;

1           Y is a phenyl or naphthyl group, or heteroaryl selected from a  
2 group consisting of pyridyl, thienyl, furyl, pyridazinyl, pyrimidinyl,  
3 pyrazinyl, thiazolyl, oxazolyl, imidazolyl and pyrazolyl, said phenyl and  
4 heteroaryl groups being optionally substituted with one or two  $R_2$   
5 groups;

6           A is  $(CH_2)_q$  where q is 0-5, lower branched chain alkyl having 3-6  
7 carbons, cycloalkyl having 3-6 carbons, alkenyl having 2-6 carbons and 1  
8 or 2 double bonds, alkynyl having 2-6 carbons and 1 or 2 triple bonds;

9           B is hydrogen, COOH or a pharmaceutically acceptable salt  
10 thereof,  $COOR_8$ ,  $CONR_9R_{10}$ ,  $-CH_2OH$ ,  $CH_2OR_{11}$ ,  $CH_2OCOR_{11}$ , CHO,  
11  $CH(OR_{12})_2$ ,  $CHOR_{13}O$ ,  $-COR_7$ ,  $CR_7(OR_{12})_2$ ,  $CR_7OR_{13}O$ , or tri-lower  
12 alkylsilyl, where  $R_7$  is an alkyl, cycloalkyl or alkenyl group containing 1  
13 to 5 carbons,  $R_8$  is an alkyl group of 1 to 10 carbons or  
14 trimethylsilylalkyl where the alkyl group has 1 to 10 carbons, or a  
15 cycloalkyl group of 5 to 10 carbons, or  $R_8$  is phenyl or lower  
16 alkylphenyl,  $R_9$  and  $R_{10}$  independently are hydrogen, an alkyl group of 1  
17 to 10 carbons, or a cycloalkyl group of 5-10 carbons, or phenyl or lower  
18 alkylphenyl,  $R_{11}$  is lower alkyl, phenyl or lower alkylphenyl,  $R_{12}$  is lower  
19 alkyl, and  $R_{13}$  is divalent alkyl radical of 2-5 carbons, and

20            $R_{14}$  is  $(R_{15})_r$ -phenyl,  $(R_{15})_r$ -naphthyl, or  $(R_{15})_r$ -heteroaryl where  
21 the heteroaryl group has 1 to 3 heteroatoms selected from the group  
22 consisting of O, S and N, r is an integer having the values of 0 - 5, and

23            $R_{15}$  is independently H, F, Cl, Br, I,  $NO_2$ ,  $N(R_8)_2$ ,  $N(R_8)COR_8$ ,  
24  $NR_8CON(R_8)_2$ , OH,  $OCOR_8$ ,  $OR_8$ , CN, an alkyl group having 1 to 10  
25 carbons, fluoro substituted alkyl group having 1 to 10 carbons, an  
26 alkenyl group having 1 to 10 carbons and 1 to 3 double bonds, alkynyl  
27 group having 1 to 10 carbons and 1 to 3 triple bonds, or a trialkylsilyl or  
28 trialkylsilyloxy group where the alkyl groups independently have 1 to 6  
29 carbons;

1  $R_{16}$  is H, lower alkyl of 1 to 6 carbons;  
2  $R_{17}$  is H, lower alkyl of 1 to 6 carbons, OH or  $OCOR_{11}$ , and  
3 p is zero or 1, with the proviso that when p is 1 then there is no  
4  $R_{17}$  substituent group, and m is an integer between 0 and 2.

5 The compounds of the present invention are useful for preventing  
6 certain undesired side effects of retinoids which are administered for the  
7 treatment or prevention of certain diseases or conditions. For this  
8 purpose the compounds of the invention may be coadministered with  
9 retinoids. The compounds of the present invention are also useful in  
10 the treatment of acute or chronic toxicity resulting from overdose or  
11 poisoning by retinoid drugs or Vitamin A.

12 The present invention additionally relates to the use of RAR  
13 antagonists for blocking all or some RAR receptor sites in biological  
14 systems, including mammals, to prevent or diminish action of RAR  
15 agonists on said receptor sites. More particularly, the present invention  
16 relates to the use of RAR antagonists for (a) the prevention and (b) the  
17 treatment of retinoid (including vitamin A or vitamin A precursor) –  
18 chronic or acute toxicity and side effects of retinoid therapy.

19 In one particular aspect of the present invention, there is  
20 provided a method of treating a pathological condition in a mammal.  
21 The conditions treated are associated with a retinoic acid receptor  
22 activity. This method involves administering to the mammal a retinoid  
23 antagonist or negative hormone capable of binding to one of the  
24 following retinoic acid receptor subtypes:  $RAR_{\alpha}$ ,  $RAR_{\beta}$  and  $RAR_{\gamma}$ .  
25 The antagonist or negative hormone is administered in an amount  
26 pharmaceutically effective to provide a therapeutic benefit against the  
27 pathological condition in the mammal.

28 As an antidote to acute or chronic retinoid or vitamin A  
29 poisoning the RAR antagonist can be administered to a mammal



1 enterally, i.e., intragastric intubation or food/water admixture, or  
2 parenterally, e.g., intraperitoneally, intramuscularly, subcutaneously,  
3 topically, etc. The only requirement for the route of administration is  
4 that it must allow delivery of the antagonist to the target tissue. The  
5 RAR antagonist can be formulated by itself or in combination with  
6 excipients. The RAR antagonist need not be in solution in the  
7 formulation, e.g., in the case of enteral use.

8 As an adjunct to therapy with retinoids and in order to prevent  
9 one or more side effects of the retinoid drug which is administered, the  
10 RAR antagonist can similarly be administered enterally or parenterally.  
11 The RAR antagonist and RAR agonist need not be administered by the  
12 same route of administration. The key is that sufficient quantities of  
13 the RAR antagonist be present continuously in the tissue of interest  
14 during exposure to the RAR agonist. For the prevention of retinoid  
15 toxicity, it is best that the RAR antagonist be administered concurrently  
16 or prior to treatment with the RAR agonist. In many situations the  
17 RAR antagonist will be administered by a different route than the  
18 agonist. For example undesirable skin effects of an enterally  
19 administered retinoid may be prevented or ameliorated by an RAR  
20 antagonist which is administered topically.

21 Another aspect of the present invention is a method of identifying  
22 retinoid negative hormones. The method includes the following steps:  
23 obtaining transfected cells containing a reporter gene transcriptionally  
24 responsive to binding of a recombinant retinoid receptor, the  
25 recombinant retinoid receptor having at least protein domains located  
26 C-terminal to a DNA binding domain of an intact retinoid receptor,  
27 measuring a basal level of reporter gene expression in untreated  
28 transfected cells, the untreated transfected cells being propagated in the  
29 absence of an added retinoid, treating the transfected cells with a

1 retinoid compound to be tested for negative hormone activity,  
2 measuring a level of reporter gene expression in treated cells,  
3 comparing the levels of reporter gene expression measured in treated  
4 cells and untreated cells, and identifying as retinoid negative hormones  
5 those retinoid compounds producing a lower level of reporter gene  
6 expression in treated cells compared with the basal level of reporter  
7 gene expression measured in untreated cells. In certain preferred  
8 embodiments of this method the intact receptor is an RAR- $\alpha$ , RAR- $\beta$   
9 or RAR- $\gamma$  subtype. In other embodiments, the intact receptor is an  
10 RXR- $\alpha$ , RXR- $\beta$  or RXR- $\gamma$  subtype. The recombinant receptor can also  
11 be either a recombinant RAR or RXR receptor. In some  
12 embodiments, the recombinant receptor is a chimeric retinoid receptor  
13 having a constitutive transcription activator domain. Such a constitutive  
14 transcription activator domain can comprise a plurality of amino acids  
15 having a net negative charge or have an amino acid sequence of a viral  
16 transcription activator domain, such as the herpes simplex virus VP-16  
17 transcription activator domain. In embodiments in which the  
18 constitutive transcription activator domain has a net negative charge, the  
19 retinoid receptor can be recombinant and have deleted therefrom a  
20 DNA binding domain, such as a DNA binding domain specific for a cis-  
21 regulatory element other than a retinoic acid responsive element. These  
22 elements include an estrogen responsive element. The transfected cell  
23 is preferably propagated in a growth medium substantially depleted of  
24 endogenous retinoids, such as one that includes activated charcoal-  
25 extracted serum. In this method, the reporter gene can be the  
26 luciferase gene, in which case, the measuring steps can involve  
27 luminometry. The reporter gene can also be the  $\beta$ -galactosidase gene,  
28 in which case the measuring steps would involve a  $\beta$ -galactosidase assay.  
29 The transfected cell can be a transfected mammalian cell, such as a

1 Green monkey cell or a human cell.

2 Another aspect of the present invention is a method of  
3 potentiating a pharmacologic activity of a steroid superfamily receptor  
4 agonist administered to a mammal. This method involves  
5 coadministering to the mammal with the steroid superfamily receptor  
6 agonist a composition comprising a pharmaceutically effective dose of a  
7 retinoid negative hormone to potentiate the pharmacologic activity of  
8 the steroid superfamily receptor agonist. The pharmacologic activity is  
9 measurable in a reporter gene *trans*-activation assay *in vitro*, such as by  
10 measuring anti-AP-1 activity. The pharmacologic activity to be  
11 potentiated can be an antiproliferative activity, such as activity of the  
12 type measurable in retinal pigment epithelium. The steroid superfamily  
13 receptor agonist can be any of the following: a retinoid receptor  
14 agonist, a vitamin D receptor agonist, a glucocorticoid receptor agonist,  
15 a thyroid hormone receptor agonist, a peroxisome proliferator-activated  
16 receptor agonist or an estrogen receptor agonist. The retinoid receptor  
17 agonist can be an RAR agonist, such as all-*trans*-retinoic acid or 13-*cis*  
18 retinoic acid. The retinoid receptor agonist can also be an RXR  
19 agonist. A preferred vitamin D receptor agonist is 1,25-  
20 dihydroxyvitamin D<sub>3</sub>. A preferred glucocorticoid receptor agonist is  
21 dexamethasone. A preferred thyroid hormone receptor agonist is 3,3',5-  
22 triiodothyronine. The retinoid negative hormone is an RAR-specific  
23 retinoid negative hormone, which preferably has a dissociation constant  
24 less than or approximately equal to 30 nM. Example of the RAR-  
25 specific retinoid negative hormone include AGN 193109, AGN 193385,  
26 AGN 193389 and AGN 193871. The composition comprising a  
27 pharmaceutically effective dose of a retinoid negative hormone can be  
28 coadministered at the same time as the steroid superfamily agonist and  
29 be combined prior to coadministration. These can also be

1 coadministered as separate compositions.

2 Brief Description of the Drawings

3 Figure 1 shows the chemical structure of AGN 193109.

4 Figures 2A - 2F are a series of line graphs showing that AGN  
5 193109 inhibited ATRA-dependent transactivation at the RARs.

6 Figures 2A and 2B represent activity at the RAR- $\alpha$  receptor; Figures 2C  
7 and 2D represent activity at the RAR- $\beta$  receptor; Figures 2E and 2F  
8 represent activity at the RAR- $\gamma$  receptor. In Figures 2A, 2C and 2E,  
9 open squares represent retinoic acid treatment and filled circles  
10 represent AGN 193109 treatment. In Figures 2B, 2D and 2F the single  
11 lines represent luciferase activity measured after treatment with  $10^{-8}$  M  
12 ATRA and variable concentrations of AGN 193109.

13 Figures 3A and 3B are line graphs representing luciferase activity  
14 detected in CV-1 cells transfected with reporter plasmid ERE-tk-Luc  
15 and expression plasmid ER-RAR- $\alpha$  and stimulated with ATRA (Figure  
16 3A) or AGN 193109 (Figure 3B) at various concentrations. Data points  
17 represent the mean  $\pm$  SEM of three independent luciferase  
18 determinations. The results of transfections carried out using different  
19 amounts of co-transfected ER-RAR- $\alpha$  (0.05, 0.1 and 0.2  $\mu$ g/well) are  
20 indicated in each figure.

21 Figures 4A and 4B are line graphs representing luciferase activity  
22 in CV-1 cells transfected with reporter plasmid ERE-tk-Luc and  
23 expression plasmid ER-RAR- $\beta$  and stimulated with ATRA (Figure 4A)  
24 or AGN 193109 (Figure 4B) at various concentrations. Data points  
25 represent the mean  $\pm$  SEM of three independent luciferase  
26 determinations. The results of transfections carried out using different  
27 amounts of co-transfected ER-RAR- $\beta$  (0.05, 0.1 and 0.2  $\mu$ g/well) are  
28 indicated in each figure.

29 Figures 5A and 5B are line graphs representing luciferase activity

1 detected in CV-1 cells transfected with reporter plasmid ERE-tk-Luc  
2 and expression plasmid ER-RAR- $\gamma$  and stimulated with ATRA (Figure  
3 5A) or AGN 193109 (Figure 5B) at various concentrations. Data points  
4 represent the mean  $\pm$  SEM of three independent luciferase  
5 determinations. The results of transfections carried out using different  
6 amounts of co-transfected ER-RAR- $\gamma$  (0.05, 0.1 and 0.2  $\mu$ g/well) are  
7 indicated in each figure.

8 Figure 6 shows ATRA and AGN 193109 dose responses of CV-1  
9 cells cotransfected with the ERE-tk-Luc reporter plasmid and either the  
10 ER-RXR- $\alpha$  chimeric receptor expression plasmid alone, or in  
11 combination with the RAR- $\gamma$ -VP-16 expression plasmid. ER-RXR- $\alpha$   
12 cotransfected cells were treated with ATRA (square) and AGN 193109  
13 (diamond). Cells cotransfected with the combination of ER-RXR- $\alpha$  and  
14 RAR- $\gamma$ -VP-16 were treated with ATRA (circle) or AGN 193109  
15 (triangle).

16 Figure 7 shows a line graph representing luciferase activity  
17 measurements recorded in lysates of CV-1 cells transfected with the  
18 ERE-tk-Luc reporter and ER-RAR- $\gamma$  expression construct and then  
19 treated with ATRA at  $10^{-8}$  M and the test compounds at the  
20 concentrations indicated on the horizontal axis. The test compounds  
21 were AGN 193109 (square), AGN 193357 (open diamond), AGN  
22 193385 (circle), AGN 193389 (triangle), AGN 193840 (hatched square)  
23 and AGN 192870 (filled diamond).

24 Figure 8 shows a line graph representing luciferase activity  
25 measurements recorded in lysates of CV-1 cells transfected with the  
26 ERE-tk-Luc reporter and RAR- $\gamma$ -VP-16 and ER-RXR- $\alpha$  expression  
27 constructs and then treated with the test compounds at the  
28 concentrations indicated on the horizontal axis. The test compounds  
29 were ATRA (open square), AGN 193109 (open circle), AGN 193174

1 (open triangle), AGN 193199 (hatched square), AGN 193385 (hatched  
2 circle), AGN 193389 (inverted triangle), AGN 193840 (diagonally filled  
3 square) and AGN 193871 (half-filled diamond).

4        Figures 9A, 9B and 9C schematically diagram a mechanism  
5 whereby AGN 193109 can modulate the interaction between the RAR  
6 (shaded box) and negative coactivator proteins (-) illustrated in the  
7 context of a transactivation assay. Figure 9A shows that negative  
8 coactivator proteins and positive coactivator proteins (+) are in a  
9 binding equilibrium with the RAR. In the absence of a ligand, basal  
10 level transcription of the reporter gene results. As illustrated in Figure  
11 9B, addition of an RAR agonist promotes the association of positive  
12 coactivator proteins with the RAR and results in upregulated reporter  
13 gene transcription. As illustrated in Figure 9C, addition of AGN  
14 193109 promotes the association of negative coactivator proteins with  
15 the RAR and prevents reporter gene transcription.

16        Figure 10 is a bar graph showing the inhibition of TPA-induced  
17 Str-AP1-CAT expression as a function of AGN 191183 concentration  
18 ( $10^{-10}$  to  $10^{-12}$  M) with the AGN 193109 concentration held constant at  
19  $10^{-8}$  M. Results from trials conducted with AGN 191183 alone are  
20 shown as hatched bars while stripped bars represent the results from  
21 treatment with the combination of AGN 193109 and AGN 191183.

22        Figure 11 schematically diagrams a mechanism whereby AGN  
23 193109 can potentiate the activities of RARs and other nuclear receptor  
24 family members. As illustrated in the diagram, introduced RARs (open  
25 rectangles having AB-C-DEF domains) have increased sensitivity to  
26 RAR ligands in the anti-AP1 assay because the negative coactivator  
27 protein (ncp), present in limiting supply, is sequestered onto RARs  
28 thereby leading to two populations: RAR+ncp and RAR-ncp. RAR-  
29 ncp has increased sensitivity to ligands. Non-RAR nuclear factors

1 (shaded rectangles having AB-C-DEF domains) have increased  
2 sensitivity to cognate ligands because ncp has been sequestered to the  
3 RAR by the activity of AGN 193109. The modular domains of the  
4 nuclear receptors are designated using standard nomenclature as "AB"  
5 (ligand independent transactivation domain), "C" (DNA binding  
6 domain), and "DEF" (ligand regulated transactivation domain and  
7 dimerization domain.

8 Figure 12 is a line graph showing the effect of AGN 193109 on  
9 the 1,25-dihydroxyvitamin D<sub>3</sub> dose response in CV-1 cells transfected  
10 with the MTV-DR3-Luc reporter plasmid. Transfectants were treated  
11 with 1,25-dihydroxyvitamin D<sub>3</sub> (filled square), 1,25-dihydroxyvitamin D<sub>3</sub>  
12 and 10<sup>-8</sup> M AGN 193109 (filled triangle), and 1,25-dihydroxyvitamin D<sub>3</sub>  
13 and 10<sup>-7</sup> M AGN 193109 (filled circle).

14 Figure 13 is a bar graph showing the effect of AGN 193109 (10  
15 nM) coadministration on 1,25-dihydroxyvitamin D<sub>3</sub>-mediated inhibition  
16 of TPA induced Str-AP1-CAT activity. Filled bars represent inhibition  
17 of CAT activity in transfected cells treated with 1,25-dihydroxyvitamin  
18 D<sub>3</sub> alone. Open bars represent inhibition of CAT activity in transfected  
19 cells treated with the combination of 1,25-dihydroxyvitamin D<sub>3</sub> and  
20 AGN 193109.

21 Figure 14 is a line graph showing the effect of AGN 193109 alone  
22 and in combination with AGN 191183 on HeLa cells cotransfected with  
23 RAR-γ and the RAR responsive MTV-TREp-Luc reporter construct.  
24 Drug treatments illustrated in the graph are: AGN 193109 alone  
25 (square), AGN 193109 in combination with AGN 191183 at 10<sup>-10</sup> M  
26 (diamond) and AGN 193109 in combination with AGN 191183 at 10<sup>-9</sup>  
27 M.

28 Figure 15 is a line graph showing that ECE16-1 cells proliferated  
29 in response to EGF (filled square) but not in response to defined

1 medium alone (open circle). Cells treated with AGN 193109 alone are  
2 represented by the filled triangle. The filled circles represent results  
3 obtained for cells treated with 10 nM AGN 191183 and 0 - 1000 nM  
4 AGN 193109.

5 Figure 16 is a bar graph showing the effect of AGN 193109 on  
6 the proliferation of CaSki cells in the presence or absence of the AGN  
7 191183 retinoid agonist. All sample groups received 20 ng/ml of  
8 epidermal growth factor (EGF) with the exception of the sample  
9 propagated in defined medium (DM) alone (open bar). Stripped bars  
10 represent samples propagated in the absence of AGN 193109. Filled  
11 bars represent samples propagated in the presence of 1000 nM AGN  
12 193109. The concentrations of AGN 191183 used in the procedure are  
13 shown on the horizontal axis.

14 Figure 17 is a dose response curve showing that AGN 193109  
15 potentiated the antiproliferative activity of ATRA on retinal pigment  
16 epithelium (RPE) cells. Samples treated with ATRA alone are  
17 represented by filled squares. Samples treated with the combination of  
18 ATRA and AGN 193109 ( $10^{-7}$  M) are represented by filled circles. The  
19 ATRA concentration used for treating the various samples is given on  
20 the horizontal axis.

21 Figure 18 is a dose response curve showing that both 13-*cis*-RA  
22 and ATRA inhibited RPE cell growth, and that AGN 193109  
23 potentiated the antiproliferative activity of 13-*cis*-RA. The various  
24 sample treatments shown in the dose response included 13-*cis*-RA alone  
25 (filled square), 13-*cis*-RA in combination with AGN 193109 ( $10^{-6}$  M)  
26 (filled circle), 13-*cis*-RA in combination with AGN 193109 ( $10^{-8}$  M)  
27 (filled triangle), and ATRA (filled diamond). The concentrations of 13-  
28 *cis*-RA and ATRA used in the sample treatments are shown on the  
29 horizontal axis.



1        Figure 19 is a dose response curve showing that AGN 193109  
2        potentiated the antiproliferative activity of dexamethasone in primary  
3        RPE cell cultures. The various sample treatments shown in the dose  
4        response included ATRA (filled square), dexamethasone alone (filled  
5        circle), dexamethasone in combination with AGN 193109 ( $10^{-8}$  M) (filled  
6        triangle), and dexamethasone in combination with AGN 193109 ( $10^{-6}$  M)  
7        (filled diamond). The concentrations of dexamethasone and ATRA  
8        used in the sample treatments are shown on the horizontal axis.

9        Figure 20 is a dose response curve showing that AGN 193109  
10       potentiated the antiproliferative activity of thyroid hormone (T3) in  
11       primary RPE cell cultures. The various sample treatments shown in the  
12       dose response included ATRA (filled square), T3 alone (filled circle),  
13       T3 in combination with AGN 193109 ( $10^{-8}$  M) (filled triangle), T3 in  
14       combination with AGN 193109 ( $10^{-6}$  M) (filled diamond). The  
15       concentrations of T3 and ATRA used in the sample treatments are  
16       shown on the horizontal axis.

#### 17       Detailed Description of the Invention

##### 18       Definitions

19       For the purposes of the present invention, an RAR antagonist is  
20       defined as a chemical that binds to one or more of the RAR subtypes  
21       with a  $K_d$  of less than 1 micromolar ( $K_d < 1\mu\text{M}$ ) but which does not  
22       cause significant transcriptional activation of that RAR subtypes-  
23       regulated genes in a receptor co-transfection assay. Conventionally,  
24       antagonists are chemical agents that inhibit the activities of agonists.  
25       Thus, the activity of a receptor antagonist is conventionally measured by  
26       virtue of its ability to inhibit the activity of an agonist.

27       An RAR agonist is defined as a chemical that binds to one or  
28       more RAR receptor subtype with  $K_d$  of less than 1 micromol ( $K_d < 1$   
29        $\mu\text{M}$ ) and causes transcriptional activation of that RAR-subtype-

1 regulated genes in a receptor co-transfection assay. The term "RAR  
2 agonist" includes chemicals that may bind and/or activate other  
3 receptors in addition to RARs, e.g., RXR receptors.

4 As used herein, a negative hormone or inverse agonist is a ligand  
5 for a receptor which causes the receptor to adopt an inactive state  
6 relative to a basal state occurring in the absence of any ligand. Thus,  
7 while an antagonist can inhibit the activity of an agonist, a negative  
8 hormone is a ligand that can alter the conformation of the receptor in  
9 the absence of an agonist. The concept of a negative hormone or  
10 inverse agonist has been explored by Bond et al. in *Nature* 374:272  
11 (1995). More specifically, Bond et al. have proposed that unliganded  
12  $\beta_2$ -adrenoceptor exists in an equilibrium between an inactive  
13 conformation and a spontaneously active conformation. Agonists are  
14 proposed to stabilize the receptor in an active conformation.  
15 Conversely, inverse agonists are believed to stabilize an inactive receptor  
16 conformation. Thus, while an antagonist manifests its activity by virtue  
17 of inhibiting an agonist, a negative hormone can additionally manifest  
18 its activity in the absence of an agonist by inhibiting the spontaneous  
19 conversion of an unliganded receptor to an active conformation. Only a  
20 subset of antagonists will act as negative hormones. As disclosed  
21 herein, AGN 193109 is both an antagonist and a negative hormone. To  
22 date, no other retinoids have been shown to have negative hormone  
23 activity.

24 As used herein, coadministration of two pharmacologically active  
25 compounds refers to the delivery of two separate chemical entities,  
26 whether *in vitro* or *in vivo*. Coadministration refers to the simultaneous  
27 delivery of separate agents; to the simultaneous delivery of a mixture of  
28 agents; as well as to the delivery of one agent followed by delivery of  
29 the second agent. In all cases, agents that are coadministered are

1 intended to work in conjunction with each other.

2 The term alkyl refers to and covers any and all groups which are  
3 known as normal alkyl, branched-chain alkyl and cycloalkyl. The term  
4 alkenyl refers to and covers normal alkenyl, branch chain alkenyl and  
5 cycloalkenyl groups having one or more sites of unsaturation. Similarly,  
6 the term alkynyl refers to and covers normal alkynyl, and branch chain  
7 alkynyl groups having one or more triple bonds.

8 Lower alkyl means the above-defined broad definition of alkyl  
9 groups having 1 to 6 carbons in case of normal lower alkyl, and as  
10 applicable 3 to 6 carbons for lower branch chained and cycloalkyl  
11 groups. Lower alkenyl is defined similarly having 2 to 6 carbons for  
12 normal lower alkenyl groups, and 3 to 6 carbons for branch chained and  
13 cyclo- lower alkenyl groups. Lower alkynyl is also defined similarly,  
14 having 2 to 6 carbons for normal lower alkynyl groups, and 4 to 6  
15 carbons for branch chained lower alkynyl groups.

16 The term "ester" as used here refers to and covers any compound  
17 falling within the definition of that term as classically used in organic  
18 chemistry. It includes organic and inorganic esters. Where B (of  
19 Formula 1 or Formula 101) is  $\text{-COOH}$ , this term covers the products  
20 derived from treatment of this function with alcohols or thiols  
21 preferably with aliphatic alcohols having 1-6 carbons. Where the ester  
22 is derived from compounds where B is  $\text{-CH}_2\text{OH}$ , this term covers  
23 compounds derived from organic acids capable of forming esters  
24 including phosphorous based and sulfur based acids, or compounds of  
25 the formula  $\text{-CH}_2\text{OCOR}_{11}$  where  $\text{R}_{11}$  is any substituted or unsubstituted  
26 aliphatic, aromatic, heteroaromatic or aliphatic aromatic group,  
27 preferably with 1-6 carbons in the aliphatic portions.

28 Unless stated otherwise in this application, preferred esters are  
29 derived from the saturated aliphatic alcohols or acids of ten or fewer

1 carbon atoms or the cyclic or saturated aliphatic cyclic alcohols and  
2 acids of 5 to 10 carbon atoms. Particularly preferred aliphatic esters are  
3 those derived from lower alkyl acids and alcohols. Also preferred are  
4 the phenyl or lower alkyl phenyl esters.

5 Amides has the meaning classically accorded that term in organic  
6 chemistry. In this instance it includes the unsubstituted amides and all  
7 aliphatic and aromatic mono- and di- substituted amides. Unless stated  
8 otherwise in this application, preferred amides are the mono- and di-  
9 substituted amides derived from the saturated aliphatic radicals of ten  
10 or fewer carbon atoms or the cyclic or saturated aliphatic-cyclic radicals  
11 of 5 to 10 carbon atoms. Particularly preferred amides are those  
12 derived from substituted and unsubstituted lower alkyl amines. Also  
13 preferred are mono- and disubstituted amides derived from the  
14 substituted and unsubstituted phenyl or lower alkylphenyl amines.  
15 Unsubstituted amides are also preferred.

16 Acetals and ketals include the radicals of the formula-CK where  
17 K is  $(-OR)_2$ . Here, R is lower alkyl. Also, K may be  $-OR_1O-$  where R,  
18 is lower alkyl of 2-5 carbon atoms, straight chain or branched.

19 A pharmaceutically acceptable salt may be prepared for any  
20 compounds in this invention having a functionality capable of forming a  
21 salt, for example an acid functionality. A pharmaceutically acceptable  
22 salt is any salt which retains the activity of the parent compound and  
23 does not impart any deleterious or untoward effect on the subject to  
24 which it is administered and in the context in which it is administered.

25 Pharmaceutically acceptable salts may be derived from organic or  
26 inorganic bases. The salt may be a mono or polyvalent ion. Of  
27 particular interest are the inorganic ions, sodium, potassium, calcium,  
28 and magnesium. Organic salts may be made with amines, particularly  
29 ammonium salts such as mono-, di- and trialkyl amines or ethanol

1 amines. Salts may also be formed with caffeine, tromethamine and  
2 similar molecules. Where there is a nitrogen sufficiently basic as to be  
3 capable of forming acid addition salts, such may be formed with any  
4 inorganic or organic acids or alkylating agent such as methyl iodide.  
5 Preferred salts are those formed with inorganic acids such as  
6 hydrochloric acid, sulfuric acid or phosphoric acid. Any of a number of  
7 simple organic acids such as mono-, di- or tri- acid may also be used.

8 Some of the compounds of the present invention may have trans  
9 and cis (E and Z) isomers. In addition, the compounds of the present  
10 invention may contain one or more chiral centers and therefore may  
11 exist in enantiomeric and diastereomeric forms. The scope of the  
12 present invention is intended to cover all such isomers per se, as well as  
13 mixtures of cis and trans isomers, mixtures of diastereomers and racemic  
14 mixtures of enantiomers (optical isomers) as well. In the present  
15 application when no specific mention is made of the configuration (cis,  
16 trans or R or S) of a compound (or of an asymmetric carbon) then a  
17 mixture of such isomers, or either one of the isomers is intended.

18 Aryl Substituted Benzopyran, Benzothiopyran, 1,2-Dihydroquinoline and  
19 5,6-Dihydronaphthalene Derivatives Having Retinoid Antagonist  
20 Like Biological Activity

21 With reference to the symbol Y in Formula 1, the preferred  
22 compounds of the invention are those where Y is phenyl, pyridyl, thienyl  
23 or furyl. Even more preferred are compounds where Y is phenyl or  
24 pyridyl, and still more preferred where Y is phenyl. As far as  
25 substitutions on the Y (phenyl) and Y (pyridyl) groups are concerned,  
26 compounds are preferred where the phenyl group is 1,4 (para)  
27 substituted by the Z and A-B groups, and where the pyridine ring is 2,5  
28 substituted by the Z and A-B groups. (Substitution in the 2,5 positions  
29 in the "pyridine" nomenclature corresponds to substitution in the 6-

1 position in the "nicotinic acid" nomenclature.) In the preferred  
2 compounds of the invention either there is no optional  $R_2$  substituent  
3 on the Y group, or the optional  $R_2$  substituent is fluoro (F).

4 The A-B group of the preferred compounds is  $(CH_2)_n-COOH$  or  
5  $(CH_2)_n-COOR_8$ , where  $n$  and  $R_8$  are defined as above. Even more  
6 preferably  $n$  is zero and  $R_8$  is lower alkyl, or  $n$  is zero and B is  $COOH$   
7 or a pharmaceutically acceptable salt thereof.

8 In many of the presently preferred examples of compounds of the  
9 invention X is  $[C(R_1)_2]_n$  where  $n$  is 1. Compounds where  $n$  is zero  
10 (indene derivatives) and where X is S or O (benzothiopyran and  
11 benzopyran derivatives) are also preferred. When X is  $[C(R_1)_2]_n$  and  $n$   
12 is 1, then  $R_1$  preferably is alkyl of 1 to 6 carbons, even more preferably  
13 methyl.

14 The  $R_2$  group attached to the aromatic portion of the  
15 tetrahydronaphthalene, benzopyran, benzothiopyran or dihydroquinoline  
16 moiety of the compounds of Formula 1 is preferably H, F,  $CF_3$ , or  
17 alkoxy such as  $OCH_3$ .  $R_3$  is preferably hydrogen or methyl, even more  
18 preferably hydrogen.

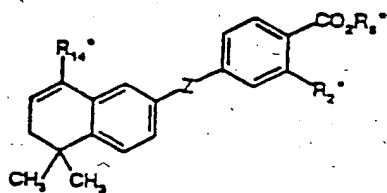
19 The  $R_2$  group attached to the Y group, when there is such  $R_2$   
20 substituent, is preferably, F or  $CF_3$ .

21 Referring now to the group Z in the compounds of the invention  
22 and shown in Formula 1, in a plurality of preferred examples Z  
23 represents an acetylenic linkage ( $Z = -C\equiv C-$ ). However, the "linker  
24 group" Z is also preferred as a diazo group ( $Z = -N=N-$ ), as an olefinic  
25 or polyolefinic group ( $Z = -(CR_1=CR_1)_n-$ ), as an ester ( $Z = -COO-$ ),  
26 amide ( $Z = -CO-NR_2-$ ) or thioamide ( $Z = -CS-NR_2-$ ) linkage.

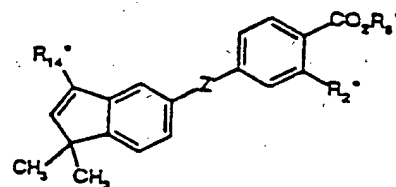
27 Referring now to the  $R_{14}$ -group, compounds are preferred where  
28  $R_{14}$  is phenyl, 2-pyridyl, 3-pyridyl, 2-thienyl, and 2-thiazolyl. The  $R_{15}$   
29 group (substituent of the  $R_{14}$  group) is preferably H, lower alkyl,

1 trifluoromethyl, chlorine, lower alkoxy or hydroxy.

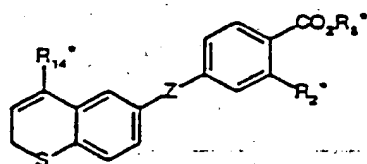
2 The presently most preferred compounds of the invention  
3 according to Formula 1 are shown in Table 1 with reference to Formula  
4 2, Formula 3, Formula 4, Formula 5, and Formula 5a.



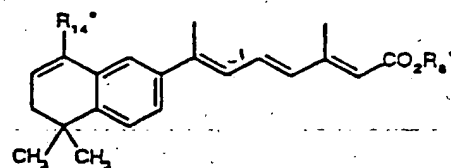
10  
11 Formula 2



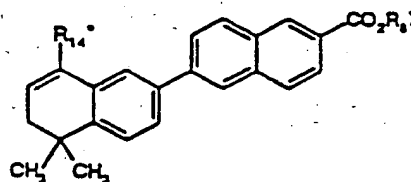
12 Formula 3;



18  
19  
20 Formula 4



21  
22  
23 Formula 5



28  
29 Formula 5a

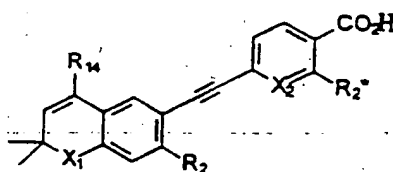
TABLE 1

1	2	3	4	5	6	7	8
	Compound	Formula	R <sub>14</sub>	Z	R <sub>2</sub>	R <sub>8</sub>	
	#						
5	1	2	4-methylphenyl	-C≡C-	H	Et	
6	1a	2	phenyl	-C≡C-	H	Et	
7	2	2	3-methylphenyl	-C≡C-	H	Et	
8	3	2	2-methylphenyl	-C≡C-	H	Et	
9	4	2	3,5-dimethylphenyl	-C≡C-	H	Et	
10	5	2	4-ethylphenyl	-C≡C-	H	Et	
11	6	2	4-t-butylphenyl	-C≡C-	H	Et	
12	7	2	4-chlorophenyl	-C≡C-	H	Et	
13	8	2	4-methoxyphenyl	-C≡C-	H	Et	
14	9	2	4-trifluoromethylphenyl	-C≡C-	H	Et	
15	10	2	2-pyridyl	-C≡C-	H	Et	
16	11	2	3-pyridyl	-C≡C-	H	Et	
17	12	2	2-methyl-5-pyridyl	-C≡C-	H	Et	
18	13	2	3-hydroxyphenyl	-C≡C-	H	Et	
19	14	2	4-hydroxy phenyl	-C≡C-	H	Et	
20	15	2	5-methyl-2-thiazolyl	-C≡C-	H	Et	
21	15a	2	2-thiazolyl	-C≡C-	H	Et	
22	16	2	4-methyl-2-thiazolyl	-C≡C-	H	Et	
23	17	2	4,5-dimethyl-2-thiazolyl	-C≡C-	H	Et	
24	18	2	2-methyl-5-pyridyl	-C≡C-	H	H	
25	19	2	2-pyridyl	-C≡C-	H	H	
26	20	2	3-methylphenyl	-C≡C-	H	H	
27	21	2	4-ethylphenyl	-C≡C-	H	H	
28	22	2	4-methoxyphenyl	-C≡C-	H	H	
29	23	2	4-trifluoromethylphenyl	-C≡C-	H	H	
30	24	2	3,5-dimethylphenyl	-C≡C-	H	H	
31	25	2	4-chlorophenyl	-C≡C-	H	H	
32	26	2	3-pyridyl	-C≡C-	H	H	
33	27	2	2-methylphenyl	-C≡C-	H	H	
34	28	2	3-hydroxyphenyl	-C≡C-	H	H	
35	29	2	4-hydroxyphenyl	-C≡C-	H	H	
36	30	2	5-methyl-2-thiazolyl	-C≡C-	H	H	
37	30a	2	2-thiazolyl	-C≡C-	H	H	
38	31	2	4-methyl-2-thiazolyl	-C≡C-	H	H	
39	32	2	4,5-dimethyl-2-thiazolyl	-C≡C-	H	H	
40	33	2	5-methyl-2-thienyl	-C≡C-	H	Et	
41	33a	2	2-thienyl	-C≡C-	H	Et	
42	34	2	5-methyl-2-thienyl	-C≡C-	H	H	
43	34a	2	2-thienyl	-C≡C-	H	H	



1	35	2	4-methylphenyl	-CONH-	H	Et
2	36	2	4-methylphenyl	-CONH-	H	H
3	37	2	4-methylphenyl	-COO-	H	Et
4	38	2	4-methylphenyl	-COO-	H	(CH <sub>2</sub> ) <sub>2</sub> Si(CH <sub>3</sub> )
5	39	2	4-methylphenyl	-COO-	H	H
6	40	2	4-methylphenyl	-CONH-	F	Et
7	41	2	4-methylphenyl	-CONH-	F	H
8	42	2	4-methylphenyl	-CSNH-	H	Et
9	43	2	4-methylphenyl	-CSNH-	H	H
10	44	2	4-methylphenyl	-CH=CH-	H	Et
11	45	2	4-methylphenyl	-CH=CH-	H	H
12	46a	2	4-methylphenyl	-N=N-	H	Et
13	46b	2	4-methylphenyl	-N=N-	H	H
14	47	3	4-methylphenyl	-C≡C-	H	Et
15	48	3	4-methylphenyl	-C≡C-	H	H
16	49	4	4-methylphenyl	-C≡C-	H	Et
17	50	4	4-methylphenyl	-C≡C-	H	H
18	51	5	4-methylphenyl	-	-	Et
19	52	5	4-methylphenyl	-	-	H
20	60	2	4-methylphenyl	-C≡C-	H	H
21	60a	2	phenyl	-C≡C-	H	H
22	61	2	4-t-butylphenyl	-C≡C-	H	H
23	62	2	4-methylphenyl	-CSNH	F	Et
24	63	2	4-methylphenyl	-CSNH	F	H
25	64	5a	4-methylphenyl	----	----	Et
26	65	5a	4-methylphenyl	----	-	H
27	66	2	2-furyl	-C≡C-	H	Et
28	67	2	2-furyl	-C≡C-	H	H

30 Still further most preferred compounds of the invention are in  
 31 accordance with Formula 5b which are further identified in Table 1a and in  
 32 the ensuing experimental section.



39 Formula 5b

Table 1a

Compound #	R <sub>14</sub>	X <sub>1</sub>	X <sub>2</sub>	R <sub>2</sub>	R <sub>2</sub> '
Page #					
242	phenyl	S	CH	H	H
243	4-methylphenyl	S	CH	H	H
244	4-ethylphenyl	S	CH	H	H
245	4- <i>i</i> -propylphenyl	S	CH	H	H
246	4- <i>t</i> -butylphenyl	S	CH	H	H
247	5-methyl-2-thienyl	S	CH	H	H
248	5-ethyl-2-thienyl	S	CH	H	H
249	5- <i>t</i> -butyl-2-thienyl	S	CH	H	H
250	6-methyl-3-pyridyl	S	CH	H	H
251	4-methylphenyl	S	CH	H	F
252	4-ethylphenyl	S	CH	H	F
253	4-methylphenyl	S	N	H	H
254	4-ethylphenyl	S	N	H	H
255	4-methylphenyl	S	CH	F	H
256	4-methylphenyl	S	CH	OCH <sub>3</sub>	H
257	5-methyl-2-thienyl	S	CH	F	H
274	phenyl	O	CH	H	H
275	4-methylphenyl	O	CH	H	H
276	4-ethylphenyl	O	CH	H	H
277	4- <i>isopropyl</i> phenyl	O	CH	H	H
278	4- <i>t</i> -butylphenyl	O	CH	H	H
279	4-methylphenyl	O	CH	H	F
280	4-ethylphenyl	O	CH	H	F

Aryl and (3-Oxo-1-Propenyl)-Substituted Benzopyran, Benzothiopyran,  
Dihydroquinoline and 5,6-Dihydronaphthalene Derivatives Having Retinoid  
Antagonist-Like Biological Activity

With reference to the symbol Y in Formula 101, the preferred compounds of the invention are those where Y is phenyl, pyridyl, thienyl or furyl. Even more preferred are compounds where Y is phenyl or pyridyl, and still more preferred where Y is phenyl. As far as substitutions on the Y (phenyl) and Y (pyridyl) groups are concerned, compounds are preferred where the phenyl group is 1,4 (para) substituted by the  $-CR_{16}=CR_{17}-$  and A-B groups, and where the pyridine ring is 2,5 substituted by the  $-CR_{16}=CR_{17}-$  and A-B groups. (Substitution in the 2,5 positions in the "pyridine" nomenclature corresponds to substitution in the 6- position in the "nicotinic acid" nomenclature.) In the preferred compounds of the invention there is no optional  $R_2$  substituent on the Y group.

The A-B group of the preferred compounds is  $(CH_2)_n-COOH$  or  $(CH_2)_n-COOR_8$ , where n and  $R_8$  are defined as above. Even more preferably n is zero and  $R_8$  is lower alkyl, or n is zero and B is  $COOH$  or a pharmaceutically acceptable salt thereof.

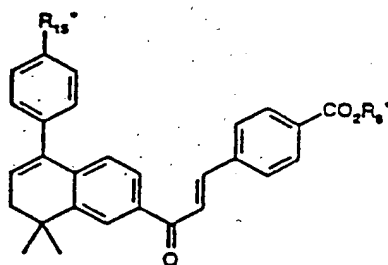
In the presently preferred examples of compounds of the invention X is  $[C(R_1)_2]_n$  where n is 1. Nevertheless, compounds where X is S or O (benzothiopyran and benzopyran derivatives) are also preferred. When X is  $[C(R_1)_2]_n$  and n is 1, then  $R_1$  preferably is alkyl of 1 to 6 carbons, even more preferably methyl.

The  $R_2$  group attached to the aromatic portion of the tetrahydronaphthalene, benzopyran, benzothiopyran or dihydroquinoline moiety of the compounds of Formula 101 is preferably H, F or  $CF_3$ .  $R_3$  is preferably hydrogen or methyl, even more preferably hydrogen.

Referring now to the  $R_{14}$  group, compounds are preferred where  $R_{14}$  is phenyl, 2-pyridyl, 3-pyridyl, 2-thienyl, and 2-thiazolyl. The  $R_{15}$  group

(substituent of the  $R_{14}$  group) is preferably H, lower alkyl, trifluoromethyl, chlorine, lower alkoxy or hydroxy.

Preferred compounds of the invention according to Formula 101 are shown in Table 2 with reference to Formula 102.



Formula 102

TABLE 2

Compound	$R_{15}$	$R_8$
101	$CH_3$	H
102	$CH_3$	Et
103	H	H
104	H	Et

#### Biological Activity, Modes of Administration

As noted above, the compounds of the present invention are antagonists of one or more RAR receptor subtypes. This means that the compounds of the invention bind to one or more RAR receptor subtypes, but do not trigger the response which is triggered by agonists of the same receptors. Some of the compounds of the present invention are antagonists of all three RAR receptor subtypes ( $RAR-\alpha$ ,  $RAR-\beta$  and  $RAR-\gamma$ ), and these are termed "RAR pan antagonists". Some others are antagonists of only one or two of RAR receptor subtypes. Some compounds within the scope of the

1 present invention are partial agonists of one or two RAR receptor subtypes  
2 and antagonists of the remaining subtypes. The compounds of the invention  
3 do not bind to RXR receptors, therefore they are neither agonists nor  
4 antagonists of RXR.

5 Depending on the site and nature of undesirable side effects which are  
6 desired to be suppressed or ameliorated, compounds used in accordance with  
7 the invention may be antagonists of only one or two of RAR receptor  
8 subtypes. Some compounds used in accordance with the invention may be  
9 partial agonists of one or two RAR receptor subtypes and antagonists of the  
10 remaining subtypes. Such compounds are, generally speaking, usable in  
11 accordance with the invention if the antagonist effect is on that RAR  
12 receptor subtype (or subtypes) which is (are) predominantly responsible for  
13 the overdose poisoning or for the undesired side effect or side effects. In this  
14 connection it is noted that, generally speaking, a compound is considered an  
15 antagonist of a given receptor subtype if in the below described co-transfection  
16 assays the compound does not cause significant transcriptional activation of  
17 the receptor regulated reporter gene, but nevertheless binds to the receptor  
18 with a  $K_d$  value of less than approximately  $1 \mu\text{M}$ .

19 Whether a compound is an RAR antagonist and therefore can be  
20 utilized in accordance with the present invention, can be tested in the  
21 following assays.

22 A chimeric receptor transactivation assay which tests for agonist-like  
23 activity in the RAR- $\alpha$ , RAR- $\beta$ , RAR- $\gamma$ , RXR- $\alpha$  receptor subtypes, and which  
24 is based on work published by Feigner P. L. and Holm M. *Focus* Vol 11, No.  
25 2 (1989) is described in detail in published PCT Application No.  
26 WO94/17796, published on August 18, 1994. The latter publication is the  
27 PCT counterpart of U. S. application serial no. 08/016,404, filed on February  
28 11, 1993, which issued as U.S. Patent No. 5,455,265. PCT publication  
29 WO94/17796 and the specification of U.S. patent 5,455,265 are hereby

1 expressly incorporated by reference. A compound should not cause  
2 significant activation of a reporter gene through a given receptor subtype  
3 (RAR- $\alpha$ , RAR- $\beta$  or RAR- $\gamma$ ) in this assay, in order to qualify as an RAR  
4 antagonist with utility in the present invention.

5 A holoreceptor transactivation assay and a ligand binding assay which  
6 measure the antagonist/agonist like activity of the compounds of the  
7 invention, or their ability to bind to the several retinoid receptor subtypes,  
8 respectively, are described in published PCT Application No. WO93/11755  
9 (particularly on pages 30 - 33 and 37 - 41) published on June 24, 1993, the  
10 specification of which is also incorporated herein by reference. A description  
11 of the holoreceptor transactivation assay is also provided below.

#### 12 Holoreceptor Transactivation Assay

13 CV1 cells (5,000 cells/well) were transfected with an RAR reporter  
14 plasmid MTV-TREp-LUC (50 ng) along with one of the RAR expression  
15 vectors (10 ng) in an automated 96-well format by the calcium phosphate  
16 procedure of Heyman et al. *Cell* 68: 397 - 406. For RXR- $\alpha$  and RXR- $\gamma$   
17 transactivation assays, an RXR-responsive reporter plasmid CRBP-II-tk-LUC  
18 (50 ng) along with the appropriate RXR expression vectors (10 ng) was used  
19 substantially as described by Heyman et al. above, and Allegretto et al. *J.*  
20 *Biol. Chem.* 268: 26625 - 26633. For RXR- $\beta$  transactivation assays, an RXR-  
21 responsive reporter plasmid CPRE-tk-LUC (50 ng) along with RXR- $\beta$   
22 expression vector (10 ng) was used as described in above. These reporters  
23 contain DRI elements from human CRBP-II and certain DRI elements from  
24 promotor, respectively (see Mangelsdorf et al. The Retinoids: Biology,  
25 Chemistry and Medicine, pp. 319 - 349, Raven Press Ltd., New York and  
26 Heyman et al., cited above). A  $\beta$ -galactosidase (50 ng) expression vector was  
27 used as an internal control in the transfections to normalize for variations in  
28 transfection efficiency. The cells were transfected in triplicate for 6 hours,  
29 followed by incubation with retinoids for 36 hours, and the extracts were

1 assayed for luciferase and  $\beta$ -galactosidase activities. The detailed  
2 experimental procedure for holoreceptor transactivations has been described  
3 in Heyman et al. above, and Allegretto et al. cited above. The results  
4 obtained in this assay are expressed in  $EC_{50}$  numbers, as they are also in the  
5 chimeric receptor transactivation assay. The Heyman et al. *Cell* 68: 397 - 406,  
6 Allegretto et al. *J. Biol. Chem.* 268: 26625 - 26633, and Mangelsdorf et al.  
7 The Retinoids: Biology, Chemistry and Medicine, pp. 319 - 349, Raven Press  
8 Ltd., New York, are expressly incorporated herein by reference. The results  
9 of ligand binding assay are expressed in  $K_d$  numbers. (See Cheng et al.  
10 *Biochemical Pharmacology* 22: 3099-3108, expressly incorporated herein by  
11 reference.)

12 Still another transactivation assay, the "PGR assay" is described in the  
13 publication Klein et al. *J. Biol. Chem.* 271, 22692-22696 (1996) which is  
14 expressly incorporated herein by reference, and a detailed description is also  
15 provided below. The results of the PGR assay are also expressed in  $EC_{50}$   
16 numbers (nanomolar concentration).

#### 17 **RAR-PGR Holoreceptor Transactivation Assay**

18 CV-1 cells ( $4 \times 10^5$  cells/well) were transiently transfected with the  
19 luciferase reporter plasmid MTV-4(R5G)-Luc (0.7 ug/well) containing four  
20 copies of the R5G retinoid DNA response element along with the RXR $\alpha$   
21 expression plasmid pRS-hRXR $\alpha$  (0.1 ug/well) and one of the RAR-P-GR  
22 expression plasmids (0.05 ug/well) in 12 well plates via calcium phosphate  
23 precipitation Chen et al. (1987) *Mol. Cell. Biol.* 7, 2745-2752 as described by  
24 Klein et al. in *J. Biol. Chem.* 271, 22692, referenced above. The three  
25 different RAR-P-GR expression plasmids, pRS-RAR $\alpha$ -P-GR, pcDNA3-  
26 RAR $\beta$ -P-GR and pcDNA3-RAR $\gamma$ -P-GR, express RAR $\alpha$ , RAR $\beta$  and RAR $\gamma$   
27 receptors, respectively, which contain modified DNA binding domains such  
28 that their "P-boxes" have been altered to that of the glucocorticoid receptor.  
29 These RAR-P-GR receptors bind to DNA as heterodimeric complexes with

1 RXR. Specifically, the RAR-P-GR receptors bind retinoic acid response  
2 elements designated R5G, comprised of two RAR half sites (nucleotide  
3 sequence 5'-GGTTCA-3') separated by 5 base pairs in which the 3'-half site  
4 has been modified to that of a glucocorticoid receptor half site, 5'-AGAACA-  
5 3'. To allow for various in transfection efficiency a  $\beta$ -galactosidase expression  
6 plasmid (0.01 ug/well) was used as an internal control. Alternatively, the  
7 assay was performed in a 96-well microtiter plate format (5000 cells/well) in a  
8 manner which was identical to that described above except 1/5 of the amount  
9 of the DNA-calcium phosphate precipitant (20  $\mu$ l instead of 100  $\mu$ l) was  
10 applied to each well. Eighteen hours after introduction of the DNA  
11 precipitants, cells were rinsed with phosphate buffered saline (PBS) and fed  
12 with D-MEM (Gibco-BRL) containing 10% activated charcoal extracted fetal  
13 bovine serum (Gemini Bio-Products). Cells were treated for 18 hours with  
14 the compounds indicated in the figures. After rinsing with PBS cells were  
15 lysed with luciferase activity was measured as previously described in *de Wet*  
16 (1987) Mol. Cell. Biol. 7, 725-737. Luciferase values represent the  
17 mean  $\pm$  SEM of triplicate determinations normalized to  $\beta$ -galactosidase  
18 activity.

19 A compound should not cause significant activation of a reporter gene  
20 through a given receptor subtype (RAR- $\alpha$ , RAR- $\beta$  or RAR- $\gamma$ ) in the  
21 transactivation assays, in order to qualify as an RAR antagonist with utility in  
22 the present invention. Last, but not least, a compound should bind to at least  
23 one of the RAR receptor subtypes in the ligand binding assay with a  $K_d$  of  
24 less than approximately 1 micromolar ( $K_d < 1 \mu$ M) in order to be capable of  
25 functioning as an antagonist of the bound receptor subtype, provided the  
26 same receptor subtype is not significantly activated by the compound.

27 Table 3 below shows the results of the holoreceptor transactivation  
28 assay for certain compounds of Table 1. Table 3a shows the results of the  
29 RAR-PGR holoreceptor transactivation assay for the preferred compounds



1 of Table 1a. Tables 4 and 4a disclose the efficacy (in percentage) in these  
 2 assays of the test compounds relative to all *trans* retinoic acid, for certain  
 3 exemplary compounds of the invention. Tables 5 and 5a show the results of  
 4 the ligand binding assay for certain exemplary compounds of the invention.

TABLE 3

Holoreceptor Transactivation Assay

Compound #	EC <sub>50</sub> (nanomolar)					
	RAR $\alpha$	RAR $\beta$	RAR $\gamma$	RXR $\alpha$	RXR $\beta$	RXR $\gamma$
18	0.00	0.00	0.00	0.00	0.00	0.00
19	0.00	0.00	0.00	0.00	0.00	0.00
20	0.00	0.00	0.00	0.00	0.00	0.00
21	0.00	0.00	0.00	0.00	0.00	0.00
22	0.00	0.00	0.00	0.00	0.00	0.00
23	0.00	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00	0.00
25	0.00	0.00	0.00	0.00	0.00	0.00
26	0.00	0.00	0.00	0.00	0.00	0.00
27	0.00	0.00	0.00	0.00	0.00	0.00
28	0.00	0.00	0.00	0.00	0.00	0.00
29	0.00	0.00	0.00	0.00	0.00	0.00
30	0.00	0.00	0.00	0.00	0.00	0.00
31	0.00	0.00	0.00	0.00	0.00	0.00
32	0.00	0.00	0.00	0.00	0.00	0.00
34	0.00	0.00	0.00	0.00	0.00	0.00
36	0.00	0.00	0.00	0.00	0.00	0.00
39	0.00	0.00	0.00	0.00	0.00	0.00
41	0.00	0.00	0.00	0.00	0.00	0.00
45	0.00	0.00	0.00	0.00	0.00	0.00
46b	0.00	0.00	0.00	0.00	0.00	0.00
52	0.00	0.00	0.00	0.00	0.00	0.00
60	0.00	0.00	0.00	0.00	0.00	0.00
61	0.00	0.00	0.00	0.00	0.00	0.00
63	0.00	0.00	0.00	0.00	0.00	0.00
101	0.00	0.00	0.00	0.00	0.00	0.00
103	0.00	0.00	0.00	0.00	0.00	0.00

37 O.O in Tables 3 and 3a indicates that the compound is less than 20 % as  
 38 active (efficacious) in this assay than all *trans* retinoic acid.

Table 3a

## RAR-PGR Holoreceptor Transactivation Assay

Compound No.	EC <sub>50</sub> (nanomolar)		
	RAR $\alpha$	RAR $\beta$	RAR $\gamma$
242	0.00	0.00	0.00
243	0.00	0.00	0.00
244	0.00	0.00	0.00
245	0.00	0.00	0.00
246	0.00	0.00	0.00
247	0.00	0.00	0.00
248	0.00	0.00	0.00
249	0.00	0.00	0.00
250	0.00	0.00	0.00
251	0.00	0.00	0.00
252	0.00	0.00	0.00
253	0.00	0.00	0.00
254	0.00	0.00	0.00
255	0.00	0.00	0.00
256	0.00	0.00	0.00
257	0.00	0.00	0.00
274	0.00	0.00	0.00
275	0.00	0.00	0.00
276	0.00	0.00	0.00
277	0.00	0.00	0.00
278	0.00	0.00	0.00
279	0.00	0.00	0.00
280	0.00	0.00	0.00

TABLE 4

Transactivation Assay Efficacy (% of RA activity)

Compound No.	RAR $\alpha$	RAR $\beta$	RAR $\gamma$	RXR $\alpha$	RXR $\beta$	RXR $\gamma$
18	4.00	1.00	0.00	2.00	10.00	1.0
19	0.00	5.0	3.0	0.0	9.0	4.0
20	3.0	4.0	0.00	4.00	0.00	3.0
21	2.00	2.00	2.00	3.00	0.00	3.00
22	0.00	0.00	2.00	1.00	0.00	2.00
23	0.00	6.00	3.00	1.00	0.00	4.00
24	3.00	7.00	4.00	1.00	0.00	3.00
25	2.00	3.00	3.00	5.00	0.00	3.00
26	1.00	6.00	0.00	2.00	0.00	3.00
27	9.00	14.00	6.00	2.00	0.00	4.00
28	2.00	10.00	2.00	2.00	0.00	3.00
29	0.00	6.00	11.00	0.00	6.00	2.00
30	3.00	5.00	1.00	0.00	9.00	3.00
31	4.00	14.00	2.00	1.00	8.00	6.00
32	0.00	2.00	2.00	1.00	0.00	2.00
34	3.00	5.00	2.00	1.00	0.00	3.00
36	1.00	5.00	0.00	1.00	7.00	2.00
39	1.00	7.00	9.00	2.00	0.00	1.00
41	3.00	5.00	6.00	1.00	0.00	3.00
45	2.00	0.00	7.00	3.00	8.00	0.00
46b	4.00	5.00	3.00	2.00	0.00	4.00
52	0.00	15.00	3.00	0.00	0.00	10.00
60	0.00	1.00	4.00	3.00	0.00	3.00
61	2.00	2.00	0.00	1.00	0.00	3.00
63	2.00	2.00	7.00	1.00	0.00	1.00
101	0.00	4.00	2.00	1.00	0.00	3.0
103	4.00	12.0	7.0	0.00	0.0	2.0

Table 4a

Compound No.	Transactivation Assay Efficacy (% of RA Activity)		
	RAR $\alpha$	RAR $\beta$	RAR $\gamma$
242	0.00	0.00	0.00
243	0.00	0.00	0.00
244	0.00	0.00	0.00
245	0.00	0.00	0.00
246	0.00	0.00	0.00
247	0.00	0.00	0.00
248	0.00	0.00	0.00
249	0.00	0.00	0.00
250	0.00	0.00	0.00
251	0.00	0.00	0.00
252	0.00	0.00	0.00
253	0.00	0.00	0.00
254	0.00	0.00	0.00
255	0.00	0.00	0.00
256	0.00	0.00	0.00
257	0.00	0.00	0.00
274	0.00	0.00	0.00
275	0.00	0.00	0.00
276	0.00	0.00	0.00
277	0.00	0.00	0.00
278	0.00	0.00	0.00
279	0.00	0.00	0.00
280	0.00	0.00	0.00

TABLE 5

Ligand Binding Assay

Compound #	K <sub>d</sub> (nanomolar)			RXR $\alpha$	RXR $\beta$	RXR $\gamma$
	RAR $\alpha$	RAR $\beta$	RAR $\gamma$			
18	24.00	11.00	24.00	0.00	0.00	0.00
19	565	210	659	0.00	0.00	0.00
20	130.00	22.0	34.00	0.00	0.00	0.00
21	16	9	13	0.00	0.00	0.00
22	24.0	17.0	27.0	0.00	0.00	0.00
23	32.00	25.00	31.00	0.00	0.00	0.00
24	699	235	286	0.00	0.00	0.00
25	50	17	20	0.00	0.00	0.00
26	40.00	31.00	36.00	0.00	0.00	0.00
27	69.00	14.00	26.00	0.00	0.00	0.00
28	669	77	236	0.00	0.00	0.00
29	234	48	80	0.00	0.00	0.00
30	683	141	219	0.00	0.00	0.00
31	370	52.00	100.00	0.00	0.00	0.00
32	0.00	89.00	169.00	0.00	0.00	0.00
34	52.00	30.00	17.00	0.00	0.00	0.00
36	13.00	550.00	0.00	0.00	0.00	0.00
39	67.00	38.00	113.00	0.00	0.00	0.00
41	5.1	491	725	0.00	0.00	0.00
45	12.0	2.80	17.0	0.00	0.00	0.00
46b	250	3.70	5.80	0.00	0.00	0.00
52	60.00	63.00	56.00	0.00	0.00	0.00
60	1.5	1.9	3.3	0.00	0.00	0.00
61	96	15	16	0.00	0.00	0.00
63	133	3219	0.00	0.00	0.00	0.00
101	750	143	637	0.00	0.00	0.00
103	301	273	261	0.00	0.00	0.00

Table 5a

Ligand Binding Assay		Kd (nanomolar)	
Compound No.	RAR $\alpha$	RAR $\beta$	RAR $\gamma$
242	8	5	13
243	4	2	10
244	3	2	5
245	7	2	10
246	13	4	20
247	12	6	18
248	6	2	17
249	19	5	57
250	4	2	17
251	2	2	3
252	4	10	4
253	45	6	5
254	70	4	9
255	3	3	3
256	>10 <sup>3</sup>	393	203
257	8	3	7
274	36	11	49
275	14	5	14
276	12	3	24
277	16	8	39
278	72	9	108
279	14	6	28
280	5	2	16

0.0 in Tables 5 and 5a indicates a value greater than 1000nM.

1       As it can be seen from the test results summarized in Tables 3, 3a, 4,  
2 4a, 5 and 5a, the therein indicated exemplary compounds of the invention are  
3 antagonists of the RAR receptor subtypes, but have no affinity to RXR  
4 receptor subtypes. (Other compounds of the invention may be antagonist of  
5 some but not all RAR receptor subtypes and agonists of the remaining RAR  
6 subtypes.) Due to this property, the compounds of the invention can be used  
7 to block the activity of RAR agonists in biological assays. In mammals,  
8 including humans, the compounds of the invention can be coadministered  
9 with RAR agonists and, by means of pharmacological selectivity or site-  
10 specific delivery, preferentially prevent the undesired effects of RAR agonists.  
11 The compounds of the invention can also be used to treat Vitamin A  
12 overdose, acute or chronic, resulting either from the excessive intake of  
13 vitamin A supplements or from the ingestion of liver of certain fish and  
14 animals that contain high level of Vitamin A. Still further, the compounds of  
15 the invention can also be used to treat acute or chronic toxicity caused by  
16 retinoid drugs. It has been known in the art that the toxicities observed with  
17 hypervitaminosis A syndrome (headache, skin peeling, bone toxicity,  
18 dyslipidemias) are similar or identical with toxicities observed with other  
19 retinoids, suggesting a common biological cause, that is RAR activation.  
20 Because the compounds of the present invention block RAR activation, they  
21 are suitable for treating the foregoing toxicities.

22       The compounds of the invention are able to substantially prevent skin  
23 irritation induced by RAR agonist retinoids, when the compound of the  
24 invention is topically coadministered to the skin. Similarly, compounds of the  
25 invention can be administered topically to the skin, to block skin irritation, in  
26 patients or animals who are administered RAR agonist compounds  
27 systemically. The compounds of the invention can accelerate recovery from  
28 pre-existing retinoid toxicity, can block hypertriglyceridemia caused by co-  
29 administered retinoids, and can block bone toxicity induced by an RAR

1 agonist (retinoid).

2       Generally speaking, for therapeutic applications in mammals in  
3 accordance with the present invention, the antagonist compounds can be  
4 administered enterally or topically as an antidote to vitamin A, vitamin A  
5 precursors, or antidote to retinoid toxicity resulting from overdose or  
6 prolonged exposure, after intake of the causative factor (vitamin A precursor  
7 or other retinoid) has been discontinued. Alternatively, the antagonist  
8 compounds are coadministered with retinoid drugs in accordance with the  
9 invention, in situations where the retinoid provides a therapeutic benefit, and  
10 where the coadministered antagonist alleviates or eliminates one or more  
11 undesired side effects of the retinoid. For this type of application the  
12 antagonist may be administered in a site-specific manner, for example as a  
13 topically applied cream or lotion while the coadministered retinoid may be  
14 given enterally.

15       For therapeutic applications in accordance with the present invention  
16 the antagonist compounds are incorporated into pharmaceutical  
17 compositions, such as tablets, pills, capsules, solutions, suspensions, creams,  
18 ointments, gels, salves, lotions and the like, using such pharmaceutically  
19 acceptable excipients and vehicles which per se are well known in the art.  
20 For example preparation of topical formulations are well described in  
21 Remington's Pharmaceutical Science, Edition 17, Mack Publishing Company,  
22 Easton, Pennsylvania. For topical application, the antagonist compounds  
23 could also be administered as a powder or spray, particularly in aerosol form.  
24 If the drug is to be administered systemically, it may be conformed as a  
25 powder, pill, tablet or the like or as a syrup or elixir suitable for oral  
26 administration. For intravenous or intraperitoneal administration, the  
27 antagonist compound will be prepared as a solution or suspension capable of  
28 being administered by injection. In certain cases, it may be useful to  
29 formulate the antagonist compounds by injection. In certain cases, it may be



1 useful to formulate the antagonist compounds in suppository form or as  
2 extended release formulation for deposit under the skin or intramuscular  
3 injection.

4 The antagonist compounds will be administered in a therapeutically  
5 effective dose in accordance with the invention. A therapeutic concentration  
6 will be that concentration which effects reduction of the particular condition  
7 (such as toxicity due to retinoid or vitamin A exposure, or side effect of  
8 retinoid drug) or retards its expansion. It should be understood that when  
9 coadministering the antagonist compounds to block retinoid-induced toxicity  
10 or side effects in accordance with the invention, the antagonist compounds  
11 are used in a prophylactic manner to prevent onset of a particular condition,  
12 such as skin irritation.

13 A useful therapeutic or prophylactic concentration will vary from  
14 condition to condition and in certain instances may vary with the severity of  
15 the condition being treated and the patient's susceptibility to treatment.  
16 Accordingly, no single concentration will be uniformly useful, but will require  
17 modification depending on the particularities of the chronic or acute retinoid  
18 toxicity or related condition being treated. Such concentrations can be  
19 arrived at through routine experimentation. However, it is anticipated that a  
20 formulation containing between 0.01 and 1.0 milligrams of antagonist  
21 compound per milliliter of formulation will constitute a therapeutically  
22 effective concentration for topical application. If administered systemically,  
23 an amount between 0.01 and 5 mg per kg per day of body weight would be  
24 expected to effect a therapeutic result.

25 The basis of the utility of RAR antagonists for the prevention or  
26 treatment of RAR agonist-induced toxicity is competitive inhibition of the  
27 activation of RAR receptors by RAR agonists. The main distinction between  
28 these two applications of RAR antagonists is the presence or absence of  
29 preexisting retinoid toxicity. Most of the examples immediately described

1 below relate to the use of retinoids to prevent retinoid toxicity, but the  
2 general methods described herein are applicable to the treatment of  
3 preexisting retinoid toxicity as well.

4 **Description of experiments demonstrating the use of RAR antagonists to**  
5 **prevent or treat retinoid toxicity and/or side effects of retinoid drugs**

6 Example 1: skin irritation induced by topically applied agonist is treated with  
7 topically applied antagonist

8 The compound 4-[(E)-2-(5,6,7,8-tetrahydro-5,5,8,8-  
9 tetramethylnaphthalen-2-yl)propen-1-yl]benzoic acid, designated AGN  
10 191183, is known in the prior art as a potent RAR agonist (see for example  
11 the descriptive portion and figure 2b of United States Patent No. 5,324,840).  
12 (The "AGN" number is an arbitrarily designated reference number utilized by  
13 the corporate assignee of the present invention for identification of  
14 compounds.)

15 4-[(5,6-dihydro-5,5-dimethyl-8-(phenyl)-2-naphthalenyl)ethynyl]benzoic  
16 acid (AGN 192869, also designated Compound 60a) is a compound the  
17 preparation of which is described below. This compound is an RAR  
18 antagonist.

19 Skin irritation induced by an RAR agonist, AGN 191183, administered  
20 topically, can be blocked by an RAR antagonist, AGN 192869, also  
21 administered topically in hairless mice.

22 More particularly skin irritation was measured on a semiquantitative  
23 scale by the daily subjective evaluation of skin flaking and abrasions. A  
24 single number, the topical irritation score, summarizes the skin irritation  
25 induced in an animal during the course of an experiment. The topical  
26 irritation score is calculated as follows. The topical irritation score is the  
27 algebraic sum of a composite flaking score and a composite abrasion score.  
28 The composite scores range from 0-9 and 0-8 for flaking and abrasions,  
29 respectively, and take into account the maximum severity, the time of onset,



1 192869, or some combination of AGN 192869 and 191183. Doses of the  
 2 respective compounds are given in Table 7. Treatments are applied to the  
 3 dorsal skin in a total volume of 4 ml/kg ( $\sim 0.1$  ml). Mice were observed daily  
 4 and scored for flaking and abrasions up to and including 3 days after the last  
 5 treatment, i.e., day 8.

TABLE 7

Experimental Design and Results, Example 1

8		Dose	Dose	Molar Ratio	Topical
9		AGN 191183	AGN 192869	(192869:	Irritation
10	<u>Group</u>	<u>(mg/kg/d)</u>	<u>(mg/kg/d)</u>	<u>(191183</u>	<u>Score)</u>
11					
12	A	0	0	--	$0 \pm 0$
13	B	0.025	0	--	$8 \pm 2$
14	C	0.025	0.06	2:1	$5 \pm 2$
15	D	0.025	0.30	10:1	$2 \pm 1$
16	E	0.025	1.5	50:1	$1 \pm 0$
17	F	0	1.5	--	$0 \pm 0$

18  
 19 The topical irritation scores for Example 1 are given in Table 7.  
 20 Neither acetone (vehicle) nor AGN 192869 (antagonist) at a dose of 1.5  
 21 mg/kg/d (group F) caused observable topical irritation. AGN 191183, the  
 22 RAR agonist, caused modest topical irritation at a dose of 0.025 mg/kg/d.  
 23 However, AGN 191183-induced topical irritation was inhibited in a dose-  
 24 dependent fashion by AGN 192869, with nearly complete abrogation of  
 25 irritation in the presence of a 50-fold molar excess of AGN 192869. This  
 26 demonstrates that a topical RAR antagonist blocks skin irritation caused by a  
 27 topical RAR agonist. Complete blockade of RAR agonist-induced skin  
 28 irritation can be achieved with lower molar ratios of antagonist to agonist  
 29 when the RAR antagonists is more potent, such as the compound 4-[(5,6-  
 30 dihydro-5,5- dimethyl-8-(4-methylphenyl)-2- naphthalenyl)ethynyl]benzoic acid  
 31 (AGN 193109, also designated in this application as Compound 60.)  
 32 Example 2: skin irritation induced by orally applied agonist is blocked with  
 33 topically applied antagonist

1       The potent RAR agonist AGN 191183 (4-[(E)-2- (5,6,7,8-  
2   tetrahydro-5,5,8,8-tetramethylnaphthalen-2- yl)propen-1-yl]benzoic acid) and  
3   the potent RAR antagonist 4-[(5,6-dihydro-5,5-dimethyl-8-(4-  
4   methylphenyl)-2-naphthalenyl)ethynyl]benzoic acid (AGN 193109, Compound  
5   60) were used in this example and body weight of the experimental animals  
6   (mice) was used as a marker of systemic RAR agonist exposure.

7       Groups of female hairless mice (8-12 weeks old, n=6) were treated by  
8   intra-gastric intubation with corn oil or AGN 191183 (0.26 mg/kg) suspended  
9   in corn oil (5 ml/kg). Mice were simultaneously treated topically on the  
10   dorsal skin with vehicle (97.6% acetone/2.4% dimethylsulfoxide) or solutions  
11   of AGN 193109 in vehicle (6 ml/kg). Specific doses for the different  
12   treatment groups are give in Table 8. Treatments were administered daily for  
13   4 consecutive days. Mice were weighed and graded for topical irritation daily  
14   as described in Example 1 up to and including 1 day after the last treatment.  
15   Percent body weight change is calculated by subtracting final body weight  
16   (day 5) from initial body weight (day 1), dividing by initial body weight, and  
17   multiplying by 100%. Topical irritation scores are calculated as described in  
18   Example 1.

19       Topical irritation scores and weight loss for the different groups are  
20   given in Table 8. Combined treatment with the topical and oral vehicles, i.e.,  
21   acetone and corn oil, respectively, caused no topical irritation or weight loss.  
22   Similarly, combined treatment with the oral vehicle and the topical antagonist  
23   AGN 193109 resulted in no topical irritation or weight loss. Oral AGN  
24   191183 by itself induced substantial weight loss and skin irritation. AGN  
25   191183-induced skin irritation was substantially reduced when combined with  
26   the lower dose of AGN 193109 and completely blocked at the higher dose of  
27   AGN 193109. AGN 191183-induced weight loss was also blocked in a dose-  
28   related fashion by topical AGN 193109, but the blockade was not complete.  
29   Thus, topical AGN 193109 preferentially blocked the dermal toxicity of AGN

1 191183. Presumably, low levels of AGN 193109 were absorbed systemically  
2 and thus partially blocked the weight loss induced by AGN 191183. However,  
3 such absorption would likely be even less in a species with less permeable  
4 skin, such as humans. Alternatively, the weight loss inhibition by AGN 193109  
5 could be due to amelioration of the AGN 191183 induced skin irritation.

6 TABLE 8

7 Experimental Design and Results, Example 2

8	Dose of Topical	Dose of Oral	% Weight	Topical
9	AGN 193109	AGN 191183	Gain or	Irritation
10	<u>Group</u>	<u>(mg/kg/d)</u>	<u>(Loss)</u>	<u>Score</u>
11				
12	A	0	1 ± 2	0 ± 0
13	B	0.26	(21 ± 6)	8 ± 1
14	C	0.26	(9 ± 5)	1 ± 1
15	D	0.26	(3 ± 5)	0 ± 1
16	E	0	3 ± 3	0 ± 0

17  
18 Thus, Example 2 demonstrates that RAR antagonists administered  
19 topically can be used to block preferentially the skin irritation induced by an  
20 RAR agonist administered orally.

21 Example 3: topically applied antagonist accelerates recovery from preexisting  
22 retinoid toxicity

23 In this example, weight loss is induced by topical treatment with the  
24 RAR agonist AGN 191183 and then the test animals are topically treated  
25 with either vehicle or the RAR antagonist AGN 193109.

26 Female hairless mice (8-12 weeks old, n=5) were treated topically with  
27 AGN 191183 (0.13 mg/kg/d) in vehicle (97.6% acetone/2.4% DMSO, 4 ml/kg)  
28 daily for 2 days. Groups of these same mice (n=5) were then treated  
29 topically either with vehicle or AGN 193109 in vehicle (4 ml/kg) daily for 3  
30 consecutive days beginning on day-3. Mice were weighed on days 1-5 and on  
31 day 8. Body weights are expressed as the mean ± SD. Means were  
32 compared statistically using an unpaired, two-tailed t-test. Differences were  
33 considered significant at P < 0.05.

TABLE 9

Results, Example 3

Treatment (days 3-5)	Body Weight (g)					
	<u>DAY 1</u>	<u>DAY 2</u>	<u>DAY 3</u>	<u>DAY 4</u>	<u>DAY 5</u>	<u>DAY 8</u>
vehicle	24.6 ± 1.5	23.9 ± 1.2	21.4 ± 1.2	20.3 ± 1.7	21.0 ± 1.4	24.7 ± 1.0
AGN 193109	23.9 ± 1.0	23.5 ± 1.2	21.4 ± 0.6	22.2 ± 0.7	22.8 ± 0.8	25.0 ± 1.1

The time course of body weights in Example 3 are given in Table 9.

Body weights of both groups of mice were lowered in parallel on days 2 and 3 as a result of AGN 191183 treatment on days 1 and 2. Body weights in the two groups were not significantly different on days 1, 2, or 3. However, AGN 193109 treatment significantly increased body weight relative to vehicle treatment on days 4 and 5. These data indicated that recovery from AGN 191183-induced body weight loss was accelerated by subsequent treatment with AGN 193109. Body weights were not significantly different between the two groups of mice on day 8, indicating that full recovery was achievable in both groups given sufficient time. Thus, RAR antagonists are effective in alleviating RAR agonist-induced toxicity even if RAR agonist-induced toxicity precedes RAR antagonist treatment, i.e., in the RAR agonist poisoning scenario.

Example 4: orally applied antagonist blocks hypertriglyceridemia induced by orally coadministered retinoid agonist

5-[(E)-2-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethylnaphthalen-2-yl)propen-1-yl]-2-thiophencarboxylic acid, is a known RAR/RXR pan-agonist (see United States Patent No. 5,324,840 column 32) and is designated AGN 191659. This compound was used orally to induce acute hypertriglyceridemia in rats, and AGN 193109 Compound 60 was coadministered orally to block the AGN 191659-induced hypertriglyceridemia.

Male Fischer rats (6-7 weeks old, n=5) were treated by intragastric intubation with corn oil (vehicle), AGN 191659, AGN 193109 or a

1 combination of AGN 191659 and AGN 193109. AGN 191659 and AGN  
2 193109 were given as fine suspensions in corn oil. The experimental design,  
3 including doses, is given in Table 10.

4 Blood was withdrawn from the inferior vena cava under carbon dioxide  
5 narcosis. Serum was separated from blood by low speed centrifugation.  
6 Total serum triglycerides (triglycerides plus glycerol) were measured with a  
7 standard spectrophotometric endpoint assay available commercially as a kit  
8 and adapted to a 96-well plate format. Serum triglyceride levels are  
9 expressed as the mean  $\pm$  SD. Means were compared statistically by one-way  
10 analysis of variance followed by Dunnett's test if significant differences were  
11 found. Differences were considered significant at  $P < 0.05$ .

12 As shown in Table 10, AGN 191659 by itself caused significant  
13 elevation of serum triglycerides relative to vehicle treatment. AGN 193109  
14 by itself did not significantly increase serum triglycerides. Importantly, the  
15 combination of AGN 193109 and AGN 191659 at molar ratios of 1:1 and 5:1  
16 reduced serum triglycerides to levels that were not significantly different from  
17 control.

18 TABLE 10

19 Experimental Design and Results, Example 4

20 <u>Group</u>	21 <u>Treatment (dose)</u>	22 <u>Serum Triglycerides (mg/dl)</u>
23 A	24 vehicle	25 55.0 $\pm$ 3.1
26 B	27 AGN 193109 (19.6 mg/kg)	28 52.4 $\pm$ 6.3
29 C	30 AGN 191659 (3.7 mg/kg)	31 122.5 $\pm$ 27.6
32 D	33 AGN 193109 (3.9 mg/kg)	34 55.7 $\pm$ 14.7
35 E	36 + AGN 191659 (3.7 mg/kg)	37 72.7 $\pm$ 8.9
38	39 AGN 193109 (19.6 mg/kg)	
39	40 + AGN 191659 (3.7 mg/kg)	

41 Example 4 demonstrates that an RAR antagonist can be used to block  
42 hypertriglyceridemia induced by a coadministered retinoid.

43 Example 5: parenterally applied antagonist blocks bone toxicity induced by  
parenterally coadministered retinoid agonist



1        Example 5 demonstrates that RAR antagonists can block bone toxicity  
2 induced by an RAR agonist. In this example, AGN 193109 is used to block  
3 premature epiphyseal plate closure caused by a coadministered RAR agonist,  
4 AGN 191183, in guinea pigs.

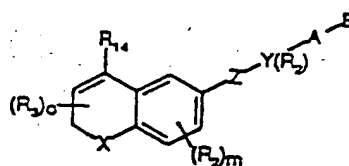
5        Groups of male Hartley guinea pigs (~ 3 weeks old, n=4) were  
6 implanted intraperitoneally with osmotic pumps containing vehicle (20%  
7 dimethylsulfoxide/80% polyethylene glycol-300), AGN 191183 (0.06 mg/ml),  
8 or AGN 191183 (0.06 mg/ml) in combination with AGN 193109 (0.34 mg/ml).  
9 The osmotic pumps are designed by the manufacturer to deliver ~ 5  $\mu$ l of  
10 solution per hour continuously for 14 days.

11        The animals were euthanized by carbon dioxide asphyxiation 14 days  
12 after implantation. The left tibia was removed and placed in 10%  
13 buffered formalin. The tibias were decalcified by exposure to a formic  
14 acid/formalin solution for 3-4 days, and paraffin sections were prepared.  
15 Sections were stained with hematoxylin and eosin by standard methods. The  
16 proximal tibial epiphyseal plate was examined and scored as closed or not  
17 closed. Epiphyseal plate closure is defined for this purpose as any  
18 interruption of the continuity of the epiphyseal growth plate cartilage, i.e.,  
19 replacement by bone and/or fibroblastic tissue.

20        None of the four vehicle-treated guinea pigs showed epiphyseal plate  
21 closure by the end of the experiment. This was expected, since the proximal  
22 epiphyseal plate of guinea pig tibia does not normally close until the animals  
23 are at least 10 months old. All four of the AGN 191183-treated guinea pigs  
24 showed partial or complete epiphyseal plate closure. However, none of the  
25 guinea pigs treated with the combination of AGN 191183 and AGN 193109  
26 demonstrated epiphyseal plate closure. Thus, AGN 193109 at a 5-fold molar  
27 excess completely blocked AGN 191183-induced bone toxicity when these  
28 compounds were coadministered parenterally.

29    RAR Antagonist Compounds

1 The compounds 4-[(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-  
 2 naphthalenyl)ethynyl]benzoic acid (AGN 193109, Compound 60) and 4-[(5,6-  
 3 dihydro-5,5-dimethyl-8-(phenyl)-2-naphthalenyl)ethynyl]benzoic acid (AGN  
 4 192869, Compound 60a) are examples of RAR antagonists which were used  
 5 in the above-described animal tests for blocking RAR receptors in  
 6 accordance with the present invention. The compounds of the following  
 7 formula (Formula 1) serve as further and general examples for additional  
 8 RAR antagonist compounds for use in accordance with the present invention.



### Formula 1

18 In Formula 1, X is S, O, NR' where R' is H or alkyl of 1 to 6 carbons,

19 or

20 X is  $[C(R_1)_2]_n$  where  $R_1$  is H or alkyl of 1 to 6 carbons, and n is an  
 21 integer between 0 or 1;

22  $R_2$  is hydrogen, lower alkyl of 1 to 6 carbons, F,  $CF_3$ , fluor substituted  
 23 alkyl of 1 to 6 carbons, OH, SH, alkoxy of 1 to 6 carbons, or alkylthio of 1 to  
 24 6 carbons;

25  $R_3$  is hydrogen, lower alkyl of 1 to 6 carbons or F;

26 m is an integer having the value of 0 - 3;

27 o is an integer having the value of 0 - 3;

28 Z is  $-C\equiv C-$ ,

29  $-N=N-$ ,

- 1            $-N=CR_1-$ ,
- 2            $-CR_1=N$ ,
- 3            $-(CR_1=CR_1)_n-$  where  $n'$  is an integer having the value 0 - 5,
- 4            $-CO-NR_1-$ ,
- 5            $-CS-NR_1-$ ,
- 6            $-NR_1-CO$ ,
- 7            $-NR_1-CS$ ,
- 8            $-COO-$ ,
- 9            $-OCO-$ ;
- 10            $-CSO-$ ;
- 11            $-OCS-$ ;

12           Y is a phenyl or naphthyl group, or heteroaryl selected from a group  
 13 consisting of pyridyl, thienyl, furyl, pyridazinyl, pyrimidinyl, pyrazinyl,  
 14 thiazolyl, oxazolyl, imidazolyl and pyrrazolyl, said phenyl and heteroaryl  
 15 groups being optionally substituted with one or two  $R_2$  groups, or

16           when Z is  $-(CR_1=CR_1)_n-$  and  $n'$  is 3, 4 or 5 then Y represents a direct  
 17 valence bond between said  $(CR_2=CR_2)_n$  group and B;

18           A is  $(CH_2)_q$  where q is 0-5, lower branched chain alkyl having 3-6  
 19 carbons, cycloalkyl having 3-6 carbons, alkenyl having 2-6 carbons and 1 or 2  
 20 double bonds, alkynyl having 2-6 carbons and 1 or 2 triple bonds;

21           B is hydrogen, COOH or a pharmaceutically acceptable salt thereof,  
 22  $COOR_8$ ,  $CONR_9R_{10}$ ,  $-CH_2OH$ ,  $CH_2OR_{11}$ ,  $CH_2OCOR_{11}$ ,  $CHO$ ,  $CH(OR_{12})_2$ ,  
 23  $CHOR_{13}O$ ,  $-COR_7$ ,  $CR_7(OR_{12})_2$ ,  $CR_7OR_{13}O$ , or tri-lower alkylsilyl, where  $R_7$   
 24 is an alkyl, cycloalkyl or alkenyl group containing 1 to 5 carbons,  $R_8$  is an  
 25 alkyl group of 1 to 10 carbons or trimethylsilylalkyl where the alkyl group has  
 26 1 to 10 carbons, or a cycloalkyl group of 5 to 10 carbons, or  $R_8$  is phenyl or  
 27 lower alkylphenyl,  $R_9$  and  $R_{10}$  independently are hydrogen, an alkyl group of  
 28 1 to 10 carbons, or a cycloalkyl group of 5-10 carbons, or phenyl or lower  
 29 alkylphenyl,  $R_{11}$  is lower alkyl, phenyl or lower alkylphenyl,  $R_{12}$  is lower alkyl,

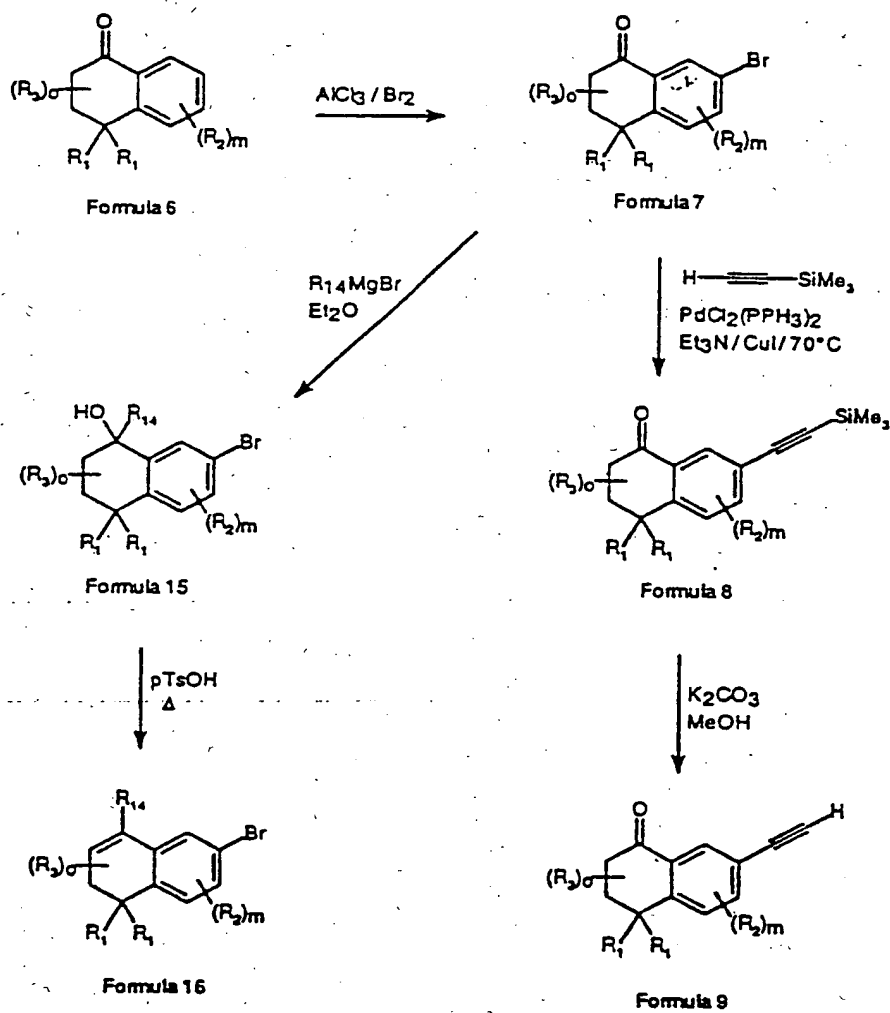
1 and  $R_{13}$  is divalent alkyl radical of 2-5 carbons, and

2  $R_{14}$  is  $(R_{15})_r$ -phenyl,  $(R_{15})_r$ -naphthyl, or  $(R_{15})_r$ -heteroaryl where the  
3 heteroaryl group has 1 to 3 heteroatoms selected from the group consisting of  
4 O, S and N,  $r$  is an integer having the values of 0 - 5, and

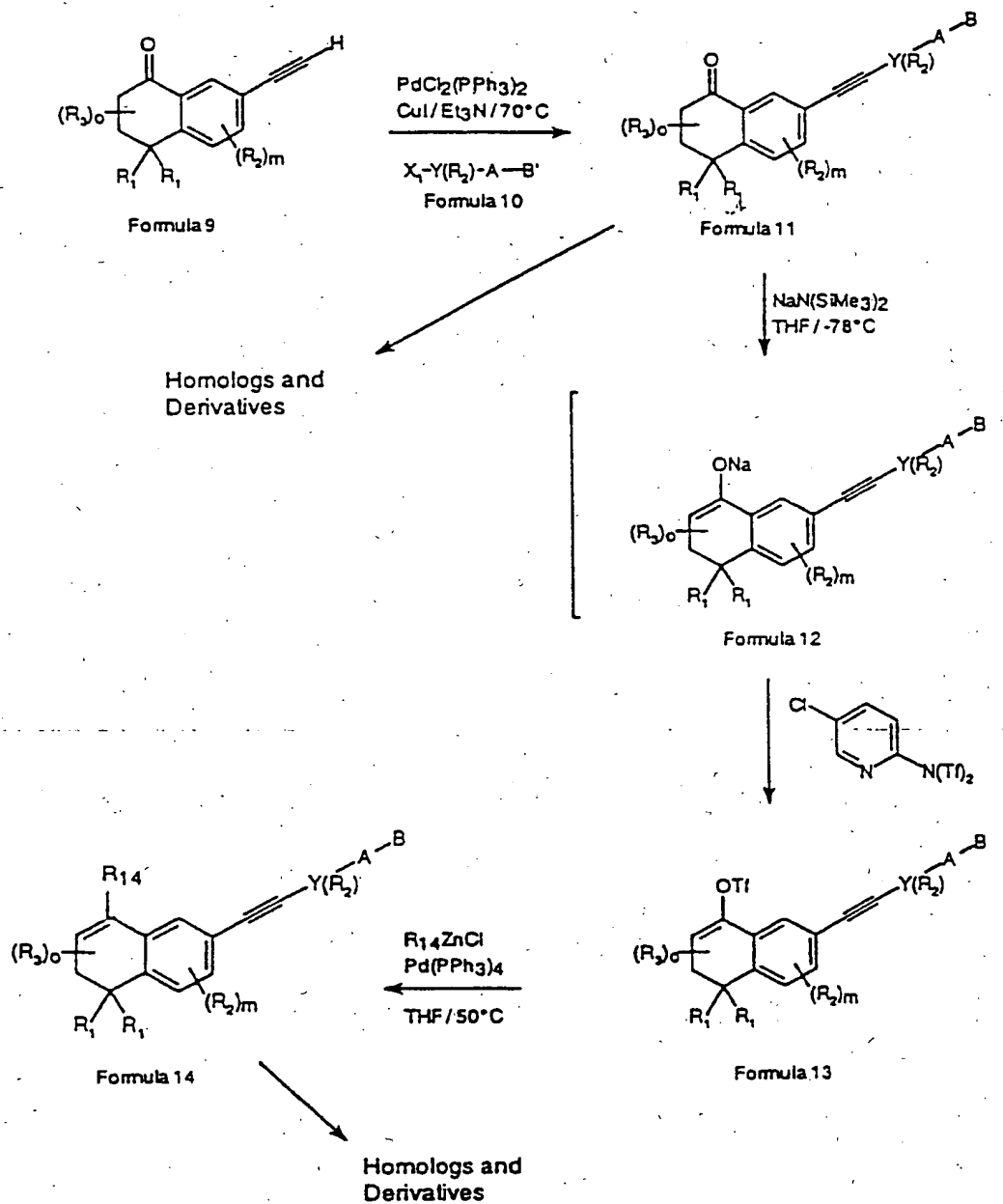
5  $R_{15}$  is independently H, F, Cl, Br, I,  $NO_2$ ,  $N(R_8)_2$ ,  $N(R_8)COR_8$ ,  
6  $NR_8CON(R_8)_2$ , OH,  $OCOR_8$ ,  $OR_8$ , CN, an alkyl group having 1 to 10  
7 carbons, fluoro substituted alkyl group having 1 to 10 carbons, an alkenyl  
8 group having 1 to 10 carbons and 1 to 3 double bonds, alkynyl group having 1  
9 to 10 carbons and 1 to 3 triple bonds, or a trialkylsilyl or trialkylsilyloxy group  
10 where the alkyl groups independently have 1 to 6 carbons.

11 Synthetic Methods - Aryl Substituted Compounds

12 The exemplary RAR antagonist compounds of Formula 1 can be made  
13 by the synthetic chemical pathways illustrated here. The synthetic chemist  
14 will readily appreciate that the conditions set out here are specific  
15 embodiments which can be generalized to any and all of the compounds  
16 represented by Formula 1.



Reaction Scheme 1



1        Reaction Scheme 1 illustrates the synthesis of compounds of Formula 1  
2        where the Z group is an ethynyl function ( $-C\equiv C-$ ) and X is  $[C(R_1)_2]_n$  where n  
3        is 1. In other words, Reaction Scheme 1 illustrates the synthesis of ethynyl  
4        substituted dihydronaphthalene derivatives of the present invention. In  
5        accordance with this scheme, a tetrahydronaphthalene-1-one compound of  
6        Formula 6 is brominated to provide the bromo derivative of Formula 7. The  
7        compounds of Formula 6 already carry the desired  $R_1$ ,  $R_2$  and  $R_3$   
8        substituents, as these are defined above in connection with Formula 1. A  
9        preferred example of a compound of Formula 6 is 3,4-dihydro-4,4-  
10       dimethyl-1(2H)-naphthalenone, which is described in the chemical literature  
11       (Arnold et al. *J. Am. Chem. Soc.* 69: 2322 - 2325 (1947)). A presently  
12       preferred route for the synthesis of this compound from 1-bromo-3-  
13       phenylpropane is also described in the experimental section of the present  
14       application.

15       The compounds of Formula 7 are then reacted with  
16       (trimethylsilyl)acetylene to provide the (trimethylsilyl)ethynyl-substituted 3,4-  
17       dihydro-naphthalen-1(2H)-one compounds of Formula 8. The reaction with  
18       (trimethylsilyl)acetylene is typically conducted under heat (approximately  
19       100°C) in the presence of cuprous iodide, a suitable catalyst, typically having  
20       the formula  $Pd(PPh_3)_2Cl_2$ , an acid acceptor (such as triethylamine) under an  
21       inert gas (argon) atmosphere. Typical reaction time is approximately 24  
22       hours. The (trimethylsilyl)ethynyl-substituted 3,4-dihydro-naphthalen-1(2H)-  
23       one compounds of Formula 8 are then reacted with base (potassium  
24       hydroxide or potassium carbonate) in an alcoholic solvent, such as methanol,  
25       to provide the ethynyl substituted 3,4-dihydro-1-naphthalen-1(2H)ones of  
26       Formula 9. Compounds of Formula 9 are then coupled with the aromatic or  
27       heteroaromatic reagent  $X_1-Y(R_2)-A-B'$  (Formula 10) in the presence of  
28       cuprous iodide, a suitable catalyst, typically  $Pd(PPh_3)_2Cl_2$ , an acid acceptor,  
29       such as triethylamine, under inert gas (argon) atmosphere. Alternatively, a

1 zinc salt (or other suitable metal salt) of the compounds of Formula 9 can be  
2 coupled with the reagents of Formula 10 in the presence of  $\text{Pd}(\text{PPh}_3)_4$  or  
3 similar complex. Typically, the coupling reaction with the reagent  $\text{X}_1\text{-Y}(\text{R}_2)\text{-}$   
4  $\text{A-B'}$  (Formula 10) is conducted at room or moderately elevated temperature.  
5 Generally speaking, coupling between an ethynylaryl derivative or its zinc salt  
6 and a halogen substituted aryl or heteroaryl compound, such as the reagent  
7 of Formula 10, is described in United States Patent No. 5,264,456, the  
8 specification of which is expressly incorporated herein by reference. The  
9 compounds of Formula 11 are precursors to exemplary compounds of the  
10 present invention, or derivatives thereof protected in the B' group, from  
11 which the protecting group can be readily removed by reactions well known  
12 in the art. The compounds of Formula 11 can also be converted into further  
13 precursors to the exemplary compounds by such reactions and  
14 transformations which are well known in the art. Such reactions are  
15 indicated in Reaction Scheme 1 by conversion into "homologs and  
16 derivatives". One such conversion employed for the synthesis of several  
17 exemplary compounds is saponification of an ester group (when B or B' is an  
18 ester) to provide the free carboxylic acid or its salt.

19       The halogen substituted aryl or heteroaryl compounds of Formula 10  
20 can, generally speaking, be obtained by reactions well known in the art. An  
21 example of such compound is ethyl 4-iodobenzoate which is obtainable, for  
22 example, by esterification of 4-iodobenzoic acid. Another example is ethyl 6-  
23 iodonicotinoate which can be obtained by conducting a halogen exchange  
24 reaction on 6-chloronicotinic acid, followed by esterification. Even more  
25 generally speaking, regarding derivatization of compounds of Formula 11  
26 and/or the synthesis of aryl and heteroaryl compounds of Formula 10 which  
27 can thereafter be reacted with compounds of Formula 9, the following well  
28 known and published general principles and synthetic methodology can be  
29 employed.



1 Carboxylic acids are typically esterified by refluxing the acid in a  
2 solution of the appropriate alcohol in the presence of an acid catalyst such as  
3 hydrogen chloride or thionyl chloride. Alternatively, the carboxylic acid can  
4 be condensed with the appropriate alcohol in the presence of  
5 dicyclohexylcarbodiimide and dimethylaminopyridine. The ester is recovered  
6 and purified by conventional means. Acetals and ketals are readily made by  
7 the method described in March, Advanced Organic Chemistry, 2nd Edition,  
8 McGraw-Hill Book Company, p. 810). Alcohols, aldehydes and ketones all  
9 may be protected by forming respectively, ethers and esters, acetals or ketals  
10 by known methods such as those described in McOmie, Plenum Publishing  
11 Press, 1973 and Protecting Groups, Ed. Greene, John Wiley & Sons, 1981.

12 To increase the value of n in the compounds of Formula 10 before  
13 affecting the coupling reaction of Reaction Scheme 1 (where such compounds  
14 corresponding to Formula 10 are not available from a commercial source)  
15 aromatic or heteroaromatic carboxylic acids are subjected to homologation by  
16 successive treatment under Arndt-Eistert conditions or other homologation  
17 procedures. Alternatively, derivatives which are not carboxylic acids may also  
18 be homologated by appropriate procedures. The homologated acids can then  
19 be esterified by the general procedure outlined in the preceding paragraph.

20 Compounds of Formula 10, (or other intermediates or exemplary  
21 compounds) where A is an alkenyl group having one or more double bonds  
22 can be made for example, by synthetic schemes well known to the practicing  
23 organic chemist; for example by Wittig and like reactions, or by introduction  
24 of a double bond by elimination of halogen from an alpha-halo-arylalkyl-  
25 carboxylic acid, ester or like carboxaldehyde. Compounds of Formula 10 (or  
26 other intermediates or exemplary compounds) where the A group has a triple  
27 (acetylenic) bond can be made by reaction of a corresponding aromatic  
28 methyl ketone with strong base, such as lithium diisopropylamide, reaction  
29 with diethyl chlorophosphate and subsequent addition of lithium

1 diisopropylamide.

2       The acids and salts derived from compounds of Formula 11 (or other  
3 intermediates or exemplary compounds) are readily obtainable from the  
4 corresponding esters. Basic saponification with an alkali metal base will  
5 provide the acid. For example, an ester of Formula 11 (or other  
6 intermediates or exemplary compounds) may be dissolved in a polar solvent  
7 such as an alkanol, preferably under an inert atmosphere at room  
8 temperature, with about a three molar excess of base, for example, lithium  
9 hydroxide or potassium hydroxide. The solution is stirred for an extended  
10 period of time, between 15 and 20 hours, cooled, acidified and the  
11 hydrolysate recovered by conventional means.

12       The amide may be formed by any appropriate amidation means known  
13 in the art from the corresponding esters or carboxylic acids. One way to  
14 prepare such compounds is to convert an acid to an acid chloride and then  
15 treat that compound with ammonium hydroxide or an appropriate amine.

16       Alcohols are made by converting the corresponding acids to the acid  
17 chloride with thionyl chloride or other means (J. March, Advanced Organic  
18 Chemistry, 2nd Edition, McGraw-Hill Book Company), then reducing the  
19 acid chloride with sodium borohydride (March, *Ibid*, p. 1124), which gives the  
20 corresponding alcohols. Alternatively, esters may be reduced with lithium  
21 aluminum hydride at reduced temperatures. Alkylating these alcohols with  
22 appropriate alkyl halides under Williamson reaction conditions (March, *Ibid*,  
23 p. 357) gives the corresponding ethers. These alcohols can be converted to  
24 esters by reacting them with appropriate acids in the presence of acid  
25 catalysts or dicyclohexylcarbodiimide and dimethylaminopyridine.

26       Aldehydes can be prepared from the corresponding primary alcohols  
27 using mild oxidizing agents such as pyridinium dichromate in methylene  
28 chloride (Corey, E. J., Schmidt, G., *Tet. Lett.* 399, 1979), or dimethyl  
29 sulfoxide/oxalyl chloride in methylene chloride (Omura, K., Swern, D.,

1 *Tetrahedron* 34: 1651 (1978)).

2 Ketones can be prepared from an appropriate aldehyde by treating the  
3 aldehyde with an alkyl Grignard reagent or similar reagent followed by  
4 oxidation.

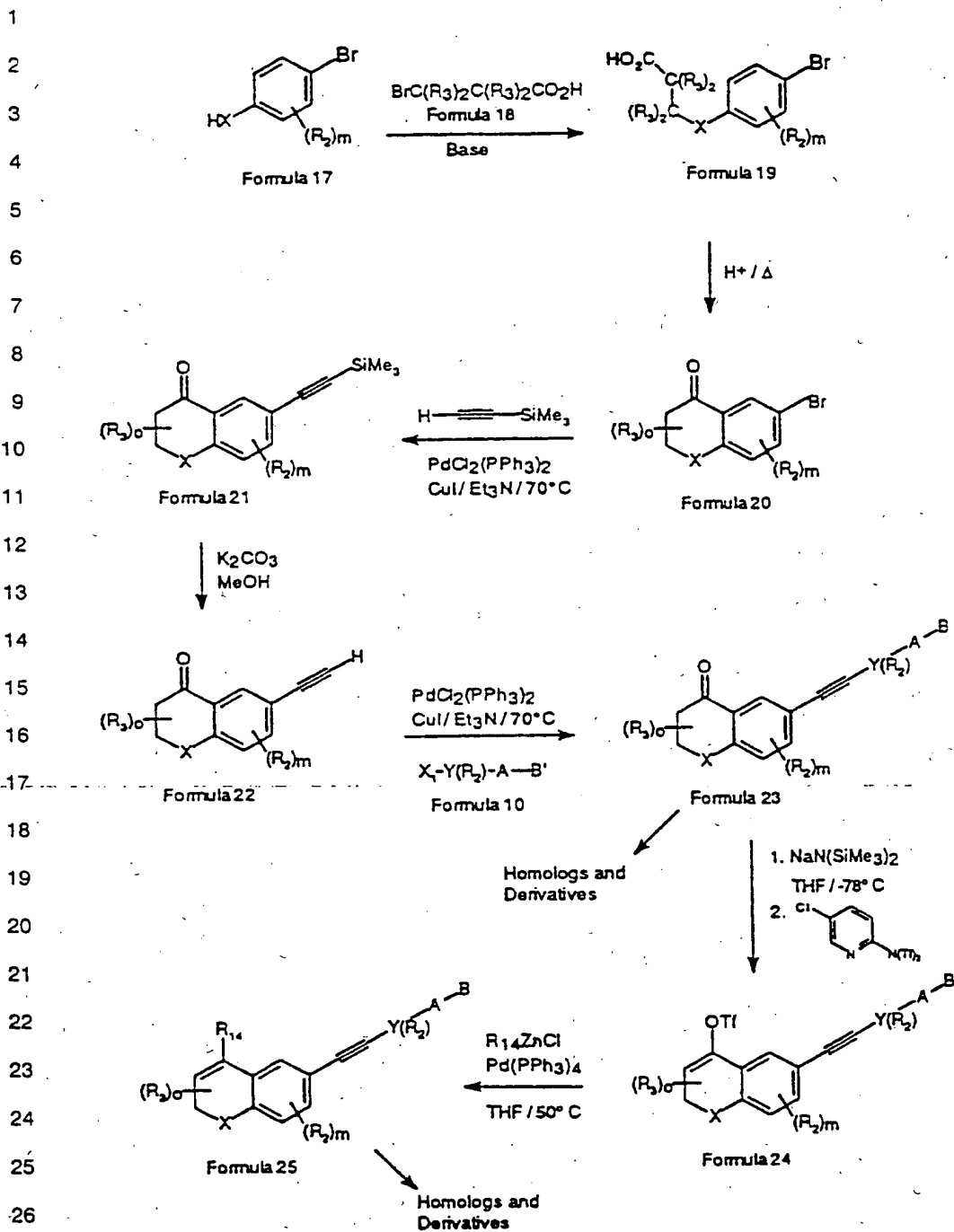
5 Acetals or ketals can be prepared from the corresponding aldehyde or  
6 ketone by the method described in March, *Ibid*, p. 810.

7 Compounds of Formula 10 (or other intermediates, or exemplary  
8 compounds) where B is H can be prepared from the corresponding  
9 halogenated aromatic or hetero aromatic compounds, preferably where the  
10 halogen is I.

11 Referring back again to Reaction Scheme 1, the compounds of  
12 Formula 11 are reacted with sodium bis(trimethylsilyl)amide and 2-[N,N-  
13 bis(trifluoromethylsulfonyl)amino]-5-chloropyridine in an inert ether type  
14 solvent, such as tetrahydrofuran, at low temperatures (-78°C and 0°C). This  
15 is shown in Reaction Scheme 1 where the usually unisolated sodium salt  
16 intermediate is shown in brackets as Formula 12. The reaction results in the  
17 trifluoromethylsulfonyloxy derivatives represented in Formula 13. (Tf =  
18 SO<sub>2</sub>CF<sub>3</sub>). The compounds of Formula 13 are then converted to the  
19 exemplary compounds of the invention, shown in Formula 14, by reaction  
20 with an organometal derivative derived from the aryl or heteroaryl compound  
21 R<sub>14</sub>H, such that the formula of the organometal derivative is R<sub>14</sub>Met (Met  
22 stands for monovalent metal), preferably R<sub>14</sub>Li. (R<sub>14</sub> is defined as in  
23 connection with Formula 1.) The reaction with the organometal derivative,  
24 preferably lithium derivative of the formula R<sub>14</sub>Li is usually conducted in an  
25 inert ether type solvent (such as tetrahydrofuran) in the presence of zinc  
26 chloride (ZnCl<sub>2</sub>) and tetrakis(triphenylphosphine)-palladium(0) (Pd(PPh<sub>3</sub>)<sub>4</sub>).  
27 The organolithium reagent R<sub>14</sub>Li, if not commercially available, can be  
28 prepared from the compound R<sub>14</sub>H (or its halogen derivative R<sub>14</sub>-X<sub>1</sub> where  
29 X<sub>1</sub> is halogen) in an ether type solvent in accordance with known practice in

1 the art. The temperature range for the reaction between the reagent  $R_{14}Li$   
2 and the compounds of Formula 13 is, generally speaking in the range of  
3 approximately  $-78^{\circ}C$  to  $50^{\circ}C$ . The compounds of Formula 14 can be  
4 converted into further homologs and derivatives in accordance with the  
5 reactions discussed above.

6 The intermediate 7-bromo-tetrahydronaphthalene-1- one compounds of  
7 Formula 7 shown in Reaction Scheme 1 can also be converted with a  
8 Grignard reagent of the formula  $R_{14}MgBr$  ( $R_{14}$  is defined as in connection  
9 with Formula 1) to yield the tertiary alcohol of Formula 15. The tertiary  
10 alcohol is dehydrated by treatment with acid to provide the 3,4-dihydro-7-  
11 bromonaphthalene derivatives of Formula 16, which serve as intermediates  
12 for the synthesis of additional compounds of the present invention (see  
13 Reaction Schemes 6, 7, and 8).



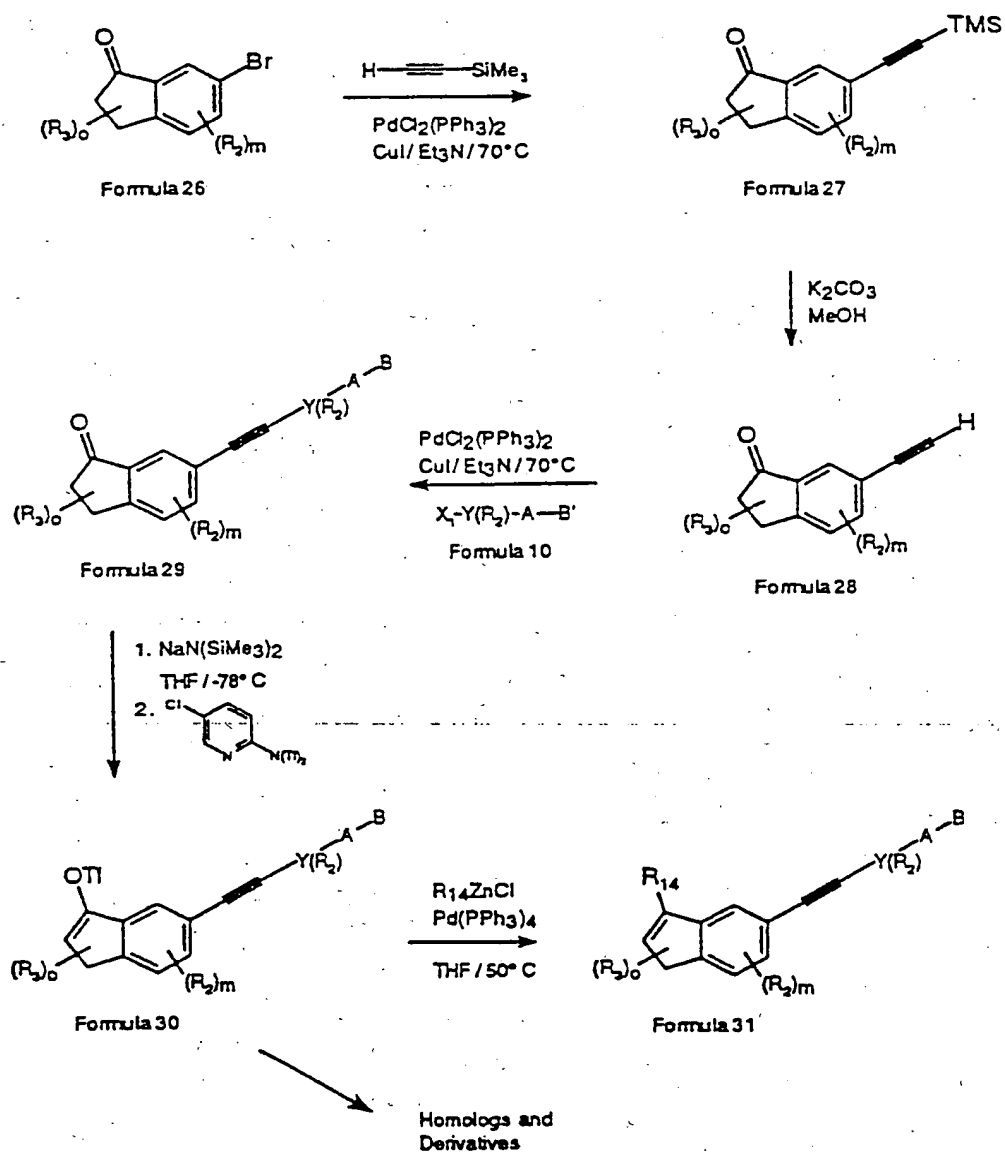
Reaction Scheme 2

1 Referring now to Reaction Scheme 2 a synthetic route to those  
2 compounds is disclosed where with reference to Formula 1 X is S, O or NR'  
3 and the Z group is an ethynyl function ( $-C\equiv C-$ ). Starting material for this  
4 sequence of the reaction is a bromophenol, bromothiophenol or bromoaniline  
5 of the structure shown in Formula 17. For the sake of simplifying the  
6 present specification, in the ensuing description X can be considered  
7 primarily sulfur as for the preparation of benzothiopyran derivatives. It  
8 should be kept in mind, however, that the herein described scheme is also  
9 suitable, with such modifications which will be readily apparent to those  
10 skilled in the art, for the preparation of benzopyran ( $X = O$ ) and  
11 dihydroquinoline ( $X = NR'$ ) compounds of the present invention. Thus, the  
12 compound of Formula 17, preferably para bromothiophenol, para  
13 bromophenol or para bromoaniline is reacted under basic condition with a 3-  
14 bromo carboxylic acid of the Formula 18. In this reaction scheme the  
15 symbols have the meaning described in connection with Formula 1. An  
16 example for the reagent of Formula 18 where  $R_3$  is hydrogen, is 3-  
17 bromopropionic acid. The reaction with the 3-bromocarboxylic acid of  
18 Formula 18 results in the compound of Formula 19. The latter is cyclized by  
19 treatment with acid to yield the 6-bromothiochroman-4-one derivative (when  
20 X is S) or 6-bromochroman derivative (when X is O) of Formula 20. The  
21 bromo compounds of Formula 20 are then subjected to substantially the same  
22 sequence of reactions under analogous conditions, which are described in  
23 Reaction Scheme 1 for the conversion of the bromo compounds of Formula  
24 7 to the compounds of the invention. Thus, briefly summarized here, the  
25 bromo compounds of Formula 20 are reacted with (trimethylsilyl)acetylene to  
26 provide the 6-(trimethylsilyl)ethynyl-substituted thiochroman-4-one or  
27 chroman-4-one compounds of Formula 21. The 6-(trimethylsilyl)ethynyl-  
28 substituted thiochroman-4-one compounds of Formula 21 are then reacted  
29 with base (potassium hydroxide or potassium carbonate) to provide the

1 ethynyl substituted 6-ethynyl substituted thiochroman-4-ones of Formula 22.  
2 Compounds of Formula 22 are then coupled with the aromatic or  
3 heteroaromatic reagent  $X_1-Y(R_2)-A-B'$  (Formula 10) under conditions  
4 analogous to those described for the analogous reactions of Reaction Scheme  
5 1, to yield the compounds of Formula 23.

6 The compounds of Formula 23 are then reacted still under conditions  
7 analogous to the similar reactions described in Reaction Scheme 1 with  
8 sodium bis(trimethylsilyl)amide and 2-[*N,N*-  
9 bis(trifluoromethylsulfonyl)amino]-5-chloropyridine to yield the 4-  
10 trifluoromethylsulfonyloxy benzothiopyran or benzopyran derivatives  
11 represented in Formula 24. The compounds of Formula 24 are then  
12 converted to compounds shown in Formula 25, by reaction with an  
13 organometal derivative derived from the aryl or heteroaryl compound  $R_{14}H$ ,  
14 as described in connection with Reaction Scheme 1.

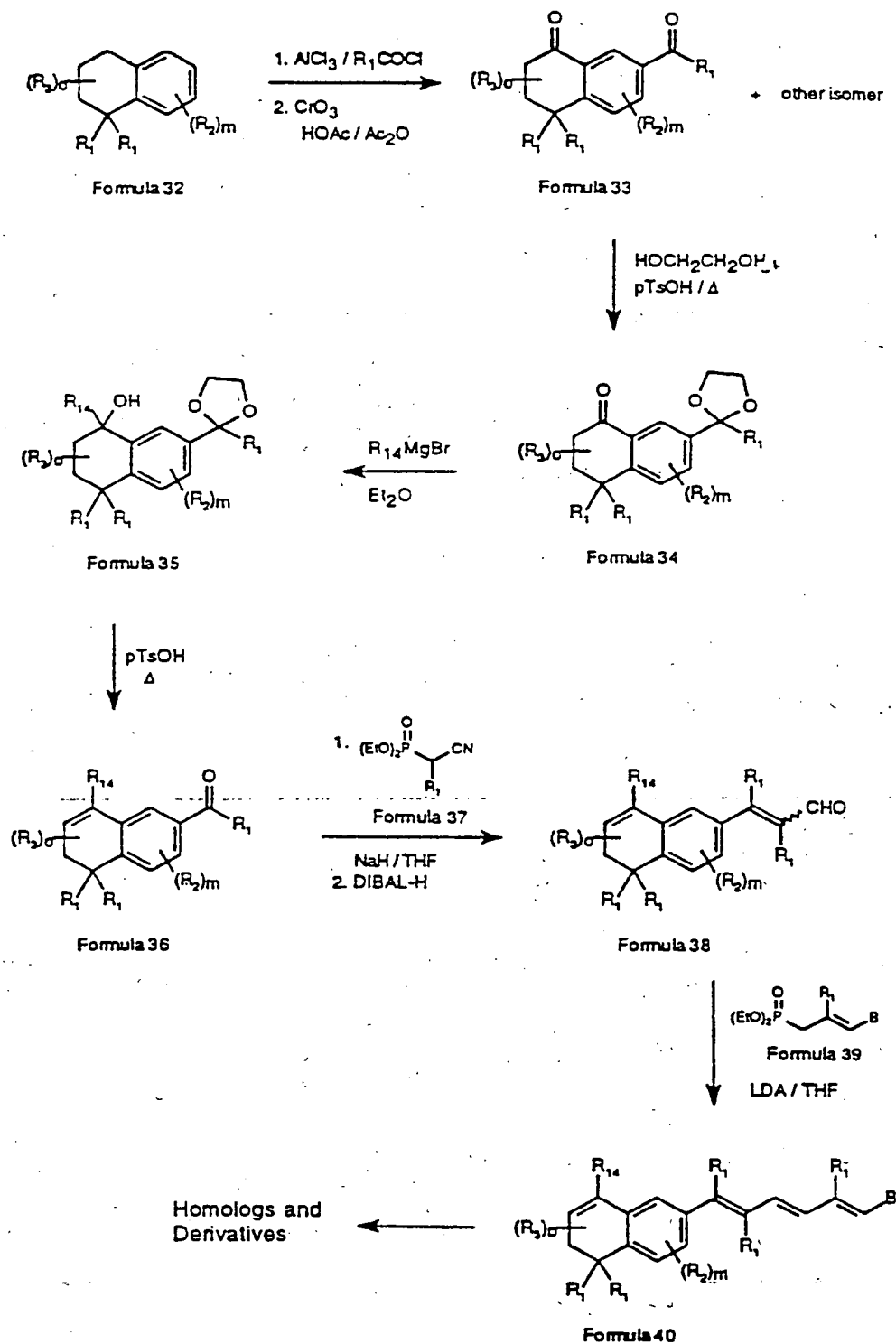
15 Similarly to the use of the intermediate 7-bromo-  
16 tetrahydronaphthalene-1-one compounds of Formula 7 of Reaction Scheme  
17 1, the intermediate 6-bromothiochroman-4-one compounds of Formula 20  
18 can also be used for the preparation of further compounds within the scope  
19 of the present invention, as described below, in Reaction Schemes 6, 7 and 8.  
20 The compounds of Formula 25, can also be converted into further homologs  
21 and derivatives, in reactions analogous to those described in connection with  
22 Reaction Scheme 1.



Reaction Scheme 3



1        Reaction Scheme 3 discloses a synthetic route to compounds where,  
2   with reference to Formula 1, X is  $[C(R_1)_2]_n$ , n is 0 and the Z group is an  
3   ethynyl function ( $-C\equiv C-$ ). In accordance with this scheme, a 6- bromo-2,3-  
4   dihydro-1H-inden-1-one derivative of Formula 26 is reacted in a sequence of  
5   reactions (starting with reaction with trimethylsilylacetylene) which are  
6   analogous to the reactions described above in connection with Reaction  
7   Schemes 1 and 2, to provide, through intermediates of the formulas 27 - 30,  
8   the indene derivatives of Formula 31. In a preferred embodiment within the  
9   scope of Reaction Scheme 3, the starting material is 6-bromo-2,3-dihydro-3,3-  
10   dimethyl-1H-inden-1-one that is available in accordance with the chemical  
11   literature (See Smith et al. Org. Prep. Proced. Int. 1978 10, 123-131).  
12   Compounds of Formula 26, such as 6-bromo-2,3-dihydro-3,3-dimethyl-1H-  
13   inden-1-one, can also be used for the synthesis of still further exemplary  
14   compounds for use in the present invention, as described below.

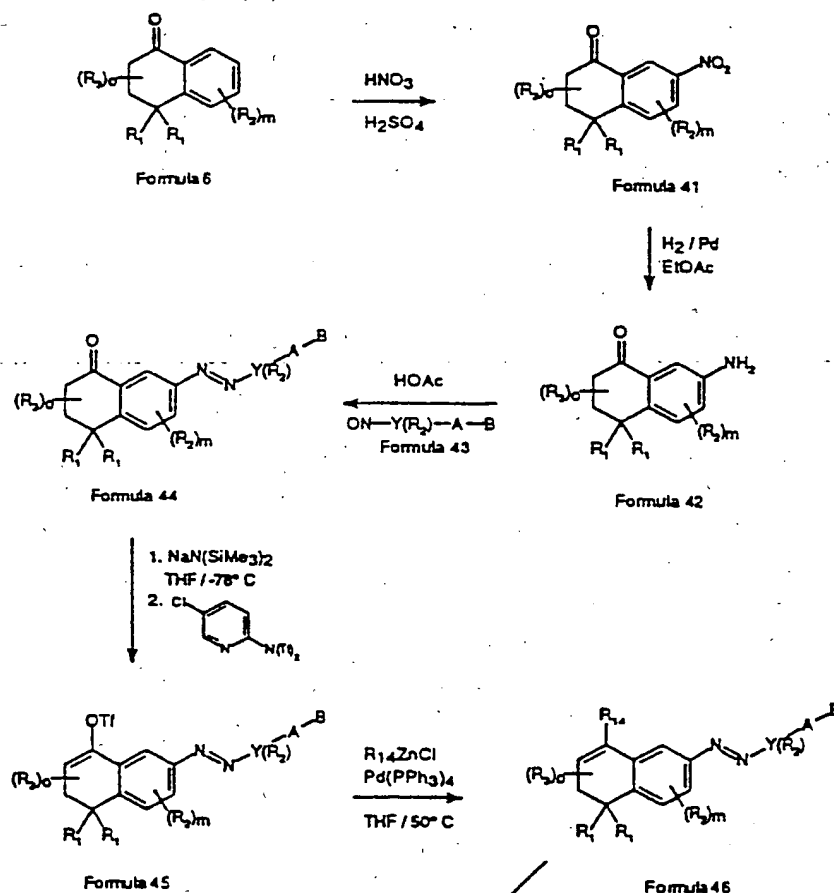


Reaction Scheme 4

1 Referring now to Reaction Scheme 4 a synthetic route to exemplary  
2 compounds is disclosed where, with reference to Formula 1, Z is -  
3  $(CR_1=CR_1)_{n'}$ ,  $n'$  is 3, 4 or 5 and Y represents a direct valence bond between  
4 the  $(CR_1=CR_1)_n$  group and B. This synthetic route is described for examples  
5 where the X group is  $[C(R_1)_2]_n$  and n is 1 (dihydronaphthalene derivatives).  
6 Nevertheless, it should be understood that the reactions and synthetic  
7 methodology described in Reaction Scheme 4 and further ensuing schemes, is  
8 also applicable, with such modifications which will be readily apparent to  
9 those skilled in the art, to derivatives where X is S, O,  $NR'$   
10 (benzothiopyran, benzopyran or dihydroquinoline derivatives) or  $[C(R_1)_2]_n$   
11 and n is 0 (indene derivatives).

12 In accordance with Reaction Scheme 4, a 1,2,3,4-  
13 tetrahydronaphthalene derivative of Formula 32 is reacted with an acid  
14 chloride ( $R_1COCl$ ) under Friedel Crafts conditions, and the resulting  
15 acetylated product is oxidized, for example in a Jones oxidation reaction, to  
16 yield a mixture of isomeric 6- and 7- acetyl-1(2H)- naphthalenone derivatives  
17 of Formula 33. In a specific preferred example of this reaction, the starting  
18 compound of Formula 32 is 1,2,3,4-tetrahydro-1,1- dimethylnaphthalene (a  
19 known compound) which can be prepared in accordance with a process  
20 described in the experimental section of the present application. The 7-  
21 acetyl-1(2H)-naphthalenone derivative of Formula 33 is reacted with ethylene  
22 glycol in the presence of acid to protect the oxo function of the exocyclic  
23 ketone moiety, as a ketal derivative of Formula 34. The ketal of Formula 34  
24 is thereafter reacted with a Grignard reagent of the formula  $R_{14}MgBr$  (the  
25 symbols are defined as in connection with Formula 1), to yield the tertiary  
26 alcohol of Formula 35. Thereafter the dioxolane protective group is removed  
27 and the tertiary alcohol is dehydrated by treatment with acid to provide the  
28 3,4- dihydro-7-acetylnaphthalene derivatives of Formula 36. The ketone  
29 function of the compounds of Formula 36 is subjected to a Horner Emmons

(or analogous) reaction under strongly alkaline conditions with a phosphonate reagent of Formula 37, to yield, after reduction, the aldehyde compounds of Formula 38. Still another Horner Emmons (or analogous) reaction under strongly alkaline conditions with a reagent of Formula 39 provides compounds of Formula 40. The latter can be converted into further homologs and derivatives in accordance with the reactions described above. A specific example of the Horner Emmons reagent of Formula 37 which is used for the preparation of a preferred compound is diethylcyanomethylphosphonate; an example of the Horner Emmons reagent of Formula 39 is diethyl-(E)-3-ethoxycarbonyl-2-methylallylphosphonate.



Reaction Scheme 5

Reaction Scheme 5 discloses a synthetic process for preparing compounds where the Z group is an azo group (-N=N-). As in Reaction Scheme 4 this process is described for examples where the X group is  $[C(R_1)_2]_n$  and n is 1 (dihydronaphthalene derivatives). Nevertheless, it should be understood that the synthetic methodology described is also applicable, with such modifications which will be readily apparent to those skilled in the art, to all azo compounds for use in the invention, namely to derivatives where X is S, O, NR' (benzothiopyran, benzopyran or dihydroquinoline derivatives) or  $[C(R_1)_2]_n$  and n is 0 (indene derivatives). Thus, a nitro group is introduced into the starting compound of Formula 6 under substantially standard conditions of nitration, to yield the 3,4-dihydro-7-nitro-1(2H)-naphthalenone derivative of Formula 41. The latter compound is reduced to the 3,4-dihydro-7-amino-1(2H)-naphthalenone derivative of Formula 42 and is thereafter reacted with a nitroso compound of the formula ON-Y(R<sub>2</sub>)-A-B (Formula 43) under conditions normally employed (glacial acetic acid) for preparing azo compounds. The nitroso compound of Formula 43 can be obtained in accordance with reactions known in the art. A specific example for such compound, which is used for the synthesis of a preferred compound is ethyl 4-nitrosobenzoate. The azo compound of Formula 44 is thereafter reacted with sodium bis(trimethylsilyl)amide and 2-[N,N-bis(trifluoromethylsulfonyl)amino]-5-chloropyridine to yield the 4-trifluoromethylsulfonyloxy derivatives represented in Formula 45. The compounds of Formula 45 are then converted to the azo compounds shown in Formula 46, by reaction with an organometallic derivative derived from the aryl or heteroaryl compound R<sub>14</sub>H. These latter two reactions, namely the conversion to the 4-trifluoromethylsulfonyloxy derivatives and subsequent reaction with the organometal derivative, have been described above in connection with Reaction Schemes 1, 2 and 3, and are employed in several presently preferred synthetic processes leading to exemplary RAR antagonist

1 compounds.

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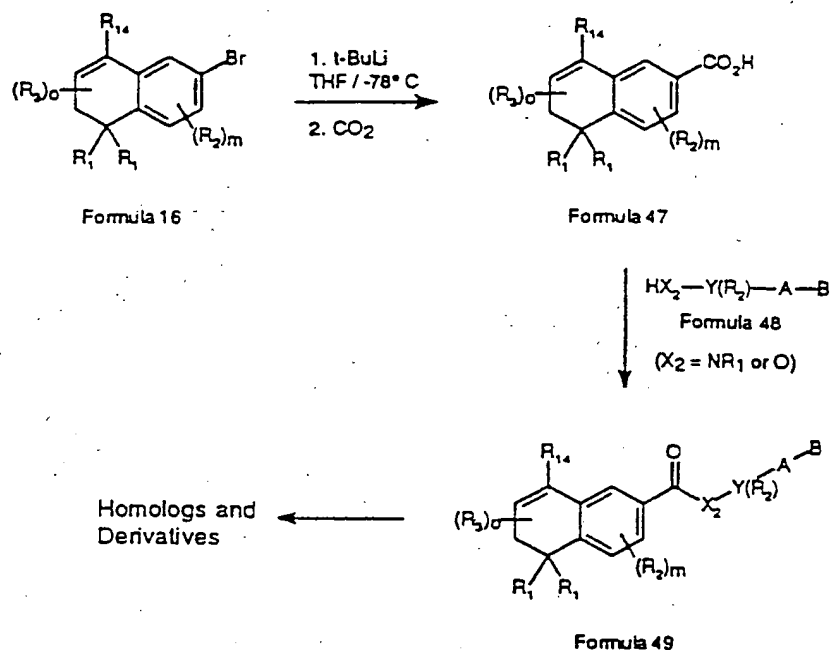
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### Reaction Scheme 6

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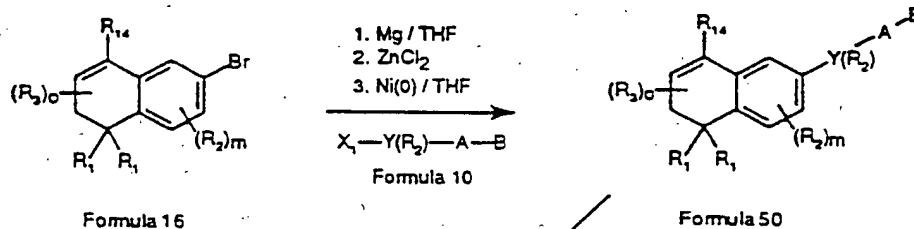
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Reaction Scheme 6 discloses a presently preferred synthetic process for the preparation of compounds where, with reference to Formula 1, the Z group is COO- or CONR<sub>1</sub> (R<sub>1</sub> is preferably H). These ester and amide derivatives are prepared from the 3,4-dihydro-7-bromonaphthalene derivatives of Formula 16, which can be obtained as described in Reaction Scheme 1. Thus, the compounds of Formula 16 are reacted with strong base, such as t-butyllithium, in an inert ether type solvent, such as tetrahydrofuran, at cold temperature, and carbon dioxide (CO<sub>2</sub>) is added to provide the 5,6-dihydro-2-naphthalenecarboxylic acid derivatives of Formula 47. Compounds of Formula 47 are then reacted with compounds of the formula X<sub>2</sub>-Y(R<sub>2</sub>)-A-B (Formula 48) where X<sub>2</sub> represent an OH or an NR<sub>1</sub> group, the R<sub>1</sub>

1 preferably being hydrogen. Those skilled in the art will recognize that the  
 2 compounds of Formula 48 are aryl or heteroaryl hydroxy or amino derivatives  
 3 which can be obtained in accordance with the state-of-the-art. The reaction  
 4 between the compounds of Formula 47 and Formula 48 can be conducted  
 5 under various known ester or amide forming conditions, such as coupling of  
 6 the two in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide  
 7 hydrochloride and 4-dimethylaminopyridine. Alternatively, the compounds  
 8 of Formula 47 can be converted into the corresponding acid chloride for  
 9 coupling with the compounds of Formula 48 in the presence of base. The  
 10 amide or ester compounds of Formula 49 can be converted into further  
 11 homologs and derivatives, as described above. Although Reaction Scheme 6  
 12 is described and shown for the example where the X group of Formula 1 is  
 13  $[C(R_1)_2]_n$  and n is 1 (dihydronaphthalene derivatives), the herein described  
 14 process can be adapted for the preparation of benzopyran, benzothiopyran,  
 15 dihydroquinoline and indene derivatives as well.

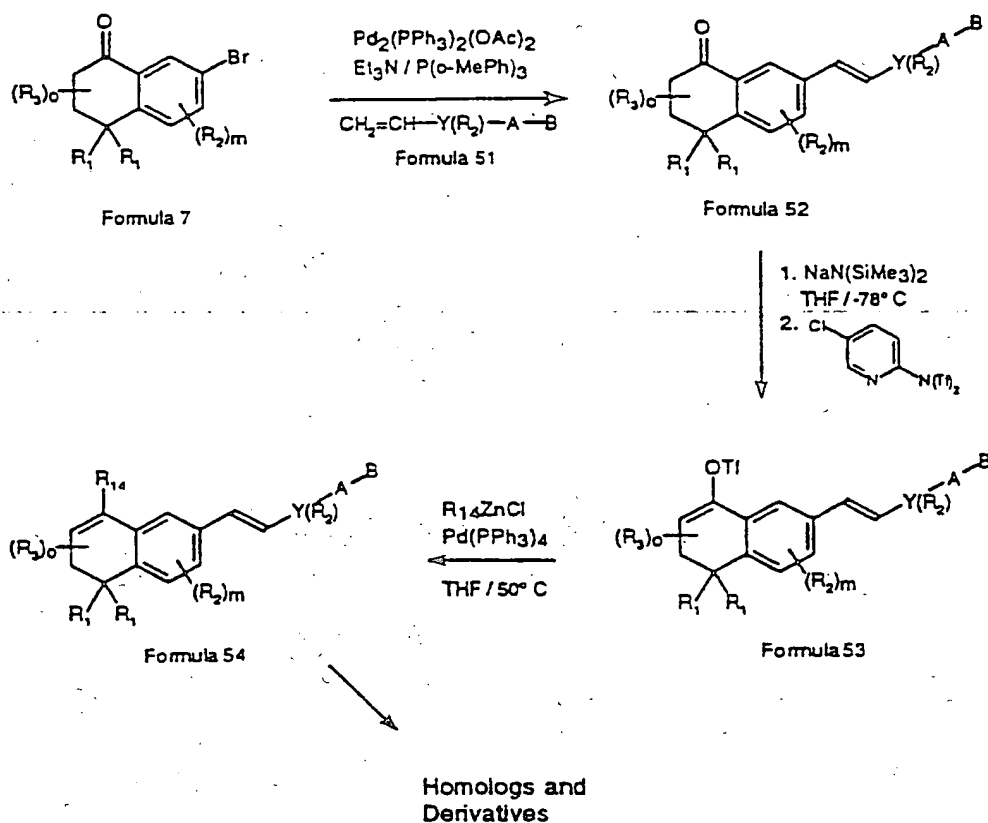
16 Compounds of the present invention where with reference to Formula  
 17 1, Z is  $-OCO-$ ,  $NR_1CO$ , as well as the corresponding thioester and thioamide  
 18 analogs, can be prepared from the intermediates derived from the  
 19 compounds of Formula 16 where the bromo function is replaced with an  
 20 amino or hydroxyl group and in accordance with the teachings of United  
 21 States Patent Nos. 5,324,744, the specification of which is expressly  
 22 incorporated herein by reference.



28 Homologs and  
 29 Derivatives

Reaction Scheme 7

Reaction Scheme 7 discloses a presently preferred synthetic process for the preparation of compounds where with reference to Formula 1, Z is -  $(CR_1=CR_1)_{n'}$  and  $n'$  is 0. These compounds of Formula 50 can be obtained in a coupling reaction between compounds of Formula 16 and a Grignard reagent derived from the halo compounds of Formula 10. The coupling reaction is typically conducted in the presence of a zinc salt and a nickel (Ni(0)) catalyst in inert ether type solvent, such as tetrahydrofuran. The compounds of Formula 50 can be converted into further homologs and derivatives, as described above.



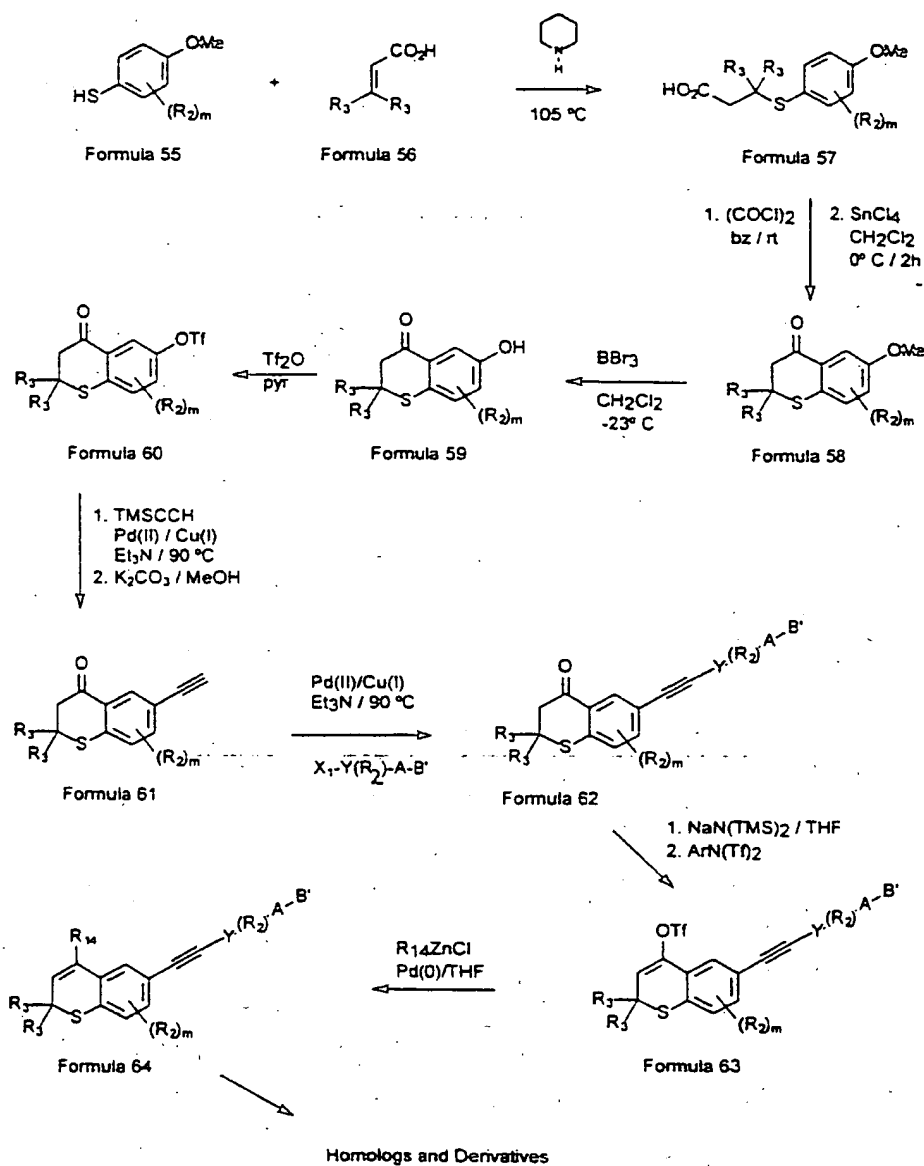
Reaction Scheme 8



1 Referring now to Reaction Scheme 8 a presently preferred synthetic  
2 process is disclosed for the preparation of compounds where Z is -  
3  $(CR_1=CR_1)_{n'}$  and  $n'$  is 1. More particularly, Reaction Scheme 8 discloses  
4 the presently preferred process for preparing those compounds which are  
5 dihydronaphthalene derivatives and where the Z group represents a vinyl (-  
6  $CH=CH-$ ) function. However, the generic methodology disclosed herein can  
7 be extended, with such modifications which will be apparent to those skilled  
8 in the art, to the analogous benzopyran, benzothiopyran, dihydroquinoline  
9 compounds, and to compounds where the vinyl group is substituted. Thus, in  
10 accordance with Reaction Scheme 8 the 7-bromo-1(2H)-naphthalenone  
11 derivative of Formula 7 is reacted with a vinyl derivative of the structure -  
12  $CH_2=CH-Y(R_2)-A-B$  (Formula 51) in the presence of a suitable catalyst,  
13 typically having the formula  $Pd(PPh_3)_4$ , an acid acceptor (such as  
14 triethylamine) under an inert gas (argon) atmosphere. The conditions of this  
15 reaction are analogous to the coupling of the acetylene derivatives of  
16 Formula 9 with the reagent of Formula 10 (see for example Reaction Scheme  
17 1), and this type of reaction is generally known in the art as a Heck reaction.  
18 The vinyl derivative of Formula 51 can be obtained in accordance with the  
19 state of the art, an example for such a reagent used for the synthesis of a  
20 preferred compound to be used in the invention is ethyl 4-vinylbenzoate.  
21 The product of the Heck coupling reaction is an ethenyl derivative of  
22 Formula 52, which is thereafter converted into compounds used in the  
23 present invention by treatment with sodium bis(trimethylsilyl)amide and 2-  
24 [*N,N*-bis(trifluoromethylsulfonyl)amino]-5-chloropyridine to yield the 4-  
25 trifluoromethylsulfonyloxy derivatives of Formula 53, and subsequent reaction  
26 with an organometal derivative derived from the aryl or heteroaryl compound  
27  $R_{14}H$ , as described above. The resulting compounds of Formula 54 can be  
28 converted into further homologs and derivatives.

29 The compounds of Formula 54 can also be obtained through synthetic

1 schemes which employ a Wittig or Horner Emmons reaction. For example,  
2 the intermediate of Formula 33 (see Reaction Scheme 4) can be reacted with  
3 a triphenylphosphonium bromide (Wittig) reagent or more preferably with a  
4 diethylphosphonate (Horner Emmons) reagent of the structure  $(\text{EtO})_2\text{PO}-$   
5  $\text{CH}_2--\text{Y}(\text{R}_2)-\text{A}-\text{B}$ , as described for analogous Horner Emmons reactions in  
6 United States Patent No. 5,324,840, the specification of which is incorporated  
7 herein by reference. The just mentioned Horner Emmons reaction provides  
8 intermediate compounds analogous in structure to Formula 52, and can be  
9 converted into compounds of Formula 54 by the sequence of reactions  
10 described in Reaction Scheme 8 for the compounds of Formula 52.



Reaction Scheme 9

1       A number of particularly preferred compounds of the invention, for  
2       example those shown in Formula 5b and Table 1a are prepared in accordance  
3       with Reaction Scheme 9. An important feature of the compounds prepared  
4       in accordance with Reaction Scheme 9 is that the group X with reference to  
5       Formula 1 is S and the 2 position of the benzothiopyrane (thiochromene)  
6       ring of the compounds is substituted with alkyl groups.

7       Referring now to Reaction Scheme 9, a 4-methoxythiophenol of  
8       Formula 55 serves as a starting material. The 4-methoxythiophenol of  
9       Formula 55 may be substituted with one or more  $R_2$  groups as defined in  
10      connection with Formula 1. In the synthesis of the preferred compounds in  
11      accordance Reaction Scheme 9 the  $R_2$  group of Formula 55 is either H,  
12      halogen such as F, or an alkoxy group, preferably methoxy. Examples for  
13      compounds of Formula 55 which are used for the synthesis of specific  
14      preferred compounds in accordance with the invention are 4-  
15      methoxythiophenol and 3-fluoro-4-methoxythiophenol. The thiophenol of  
16      Formula 55 is reacted with an alkenoic acid of Formula 56 by heating in  
17      piperidine. This reaction results in addition of the thiophenol to the double  
18      bond of the alkenoic acid (*Michael* addition) to give rise to compounds of  
19      Formula 57. The alkenoic acid of Formula 56 includes the  $R_3$  substituents  
20      which for the herein described preferred embodiments are alkyl, most  
21      preferably methyl, groups. An example for the alkenoic acid of Formula 56  
22      is 3-methyl-2-butenic acid. Reaction of thiophenols of Formula 55 with 2-  
23      alkenoic acids to synthesize 2,2-dimethyl-thiochroman-4-one derivatives is  
24      generally described by *Ferreira et al. Synthesis* 1987 p 149.

25      The aromatic acid of Formula 57 is converted to the corresponding  
26      acid chloride, for example by reaction with oxalyl chloride, and the resulting  
27      acid chloride is thereafter cyclized by heating in an inert solvent such as  
28       $CH_2Cl_2$  in the presence of stannic tetrachloride ( $SnCl_4$ ), to give rise to 2,2-  
29      disubstituted thiochroman-4-one compounds of Formula 58. The 2,2-

1 disubstituted thiochroman-4-one of Formula 58 is reacted with  
2 borontribromide to remove the methyl group from the methoxy function and  
3 to yield the 2,2-disubstituted 6-hydroxy-thiochroman-4-one of Formula 59.  
4 The hydroxy compound of Formula 59 is thereafter reacted with  
5 trifluoromethanesulfonic anhydride in anhydrous pyridine to give rise to the  
6 6- trifluoromethanesulfonyloxy derivative of Formula 60.

7 The 2,2-disubstituted 6-trifluoromethanesulfonyloxy-thiochroman-4-one  
8 of Formula 60, shown in Reaction Scheme 9, is reacted with  
9 (trimethylsilyl)acetylene to provide 2,2-disubstituted 6-(trimethylsilyl)ethynyl-  
10 thiochroman-4-one compounds which are subsequently reacted with base  
11 ( $K_2CO_3$ ) in an alcoholic solvent, such as methanol, to yield the 2,2-  
12 disubstituted 6-ethynyl-thiochroman-4-one compounds of Formula 61. The  
13 reaction with (trimethylsilyl)acetylene is typically conducted under heat  
14 (approximately  $95^\circ C$ ) in the presence of a suitable catalyst, typically having  
15 the formula  $Pd(PPh_3)_2Cl_2$ , and an acid acceptor (such as triethylamine) under  
16 an inert gas (argon) atmosphere. This reaction is analogous to the reactions  
17 of the 6-bromo-thiochroman-4-one compounds of Formula 20 with  
18 (trimethylsilyl)acetylene, shown in Reaction Scheme 2. Thereafter, the 2,2-  
19 disubstituted 6-ethynyl-thiochroman-4-one compound of Formula 61 is  
20 coupled with the aromatic or heteroaromatic reagent  $X_1-Y(R_2)-A-B'$   
21 (Formula 10, defined above) under conditions similar to those described for  
22 the analogous reactions of Reaction Scheme 1 and Reaction Scheme 2, to  
23 yield the compounds of Formula 62. In the synthesis of the herein described  
24 specific preferred examples of the compounds of Formula 62 ethyl 4-  
25 iodobenzoate, ethyl 2-fluoro-4-iodobenzoate and ethyl 6-iodonicotinate serve  
26 as examples for the reagent  $X_1-Y(R_2)-A-B'$  (Formula 10).

27 The compounds of Formula 62 are then reacted, still under conditions  
28 similar to the analogous reactions described in Reaction Scheme 1 and  
29 Reaction Scheme 2, with sodium bis(trimethylsilyl)amide and 2-[N,N-

1 bis(trifluoromethylsulfonyl)amino]-5-chloropyridine to yield the 4-  
2 trifluoromethylsulfonyloxy benzothiopyran (thiochromene) derivatives  
3 represented by Formula 63. The compounds of Formula 63 are then  
4 converted to compounds shown in Formula 64, by reaction with an  
5 organometal derivative derived from the aryl or heteroaryl compound  $R_{14}H$ ,  
6  $R_{14}Br$  or  $R_{14}I$  as described in connection with Reaction Scheme 1 and  
7 Reaction Scheme 2. Examples of reagents and conditions utilized in  
8 accordance with Reaction Scheme 9 to convert the 4-  
9 trifluoromethanesulfonyloxy compounds of Formula 63 into preferred  
10 compounds of the invention in accordance with Formula 64 are:

11 4-methylbromobenzene, tetrahydrofuran (THF), *t*-butyllithium and zinc  
12 chloride ( $ZnCl_2$ ) to make the organometal reagent, followed by reaction with  
13 the 4-trifluoromethylsulfonyloxy (triflate) derivative in the presence of  
14 tetrakis(triphenylphosphine)palladium(0) catalyst;

15 2-methylthiophene, THF, *n*-butyllithium and  $ZnCl_2$  followed by the  
16 reaction with the triflate in the presence of  
17 tetrakis(triphenylphosphine)palladium(0);

18 bromobenzene, THF, *t*-butyllithium and  $ZnCl_2$  followed by the reaction  
19 with the triflate in the presence of tetrakis(triphenylphosphine)palladium(0);

20 4-ethylbromobenzene, THF, *t*-butyllithium and  $ZnCl_2$  followed by the  
21 reaction with the triflate in the presence of  
22 tetrakis(triphenylphosphine)palladium(0);

23 4-*iso*-propylbromobenzene, THF, *t*-butyllithium and  $ZnCl_2$  followed by  
24 the reaction with the triflate in the presence of  
25 tetrakis(triphenylphosphine)palladium(0);

26 4-*t*-butylbromobenzene, THF, *t*-butyllithium and  $ZnCl_2$  followed by the  
27 reaction with the triflate in the presence of  
28 tetrakis(triphenylphosphine)palladium(0);

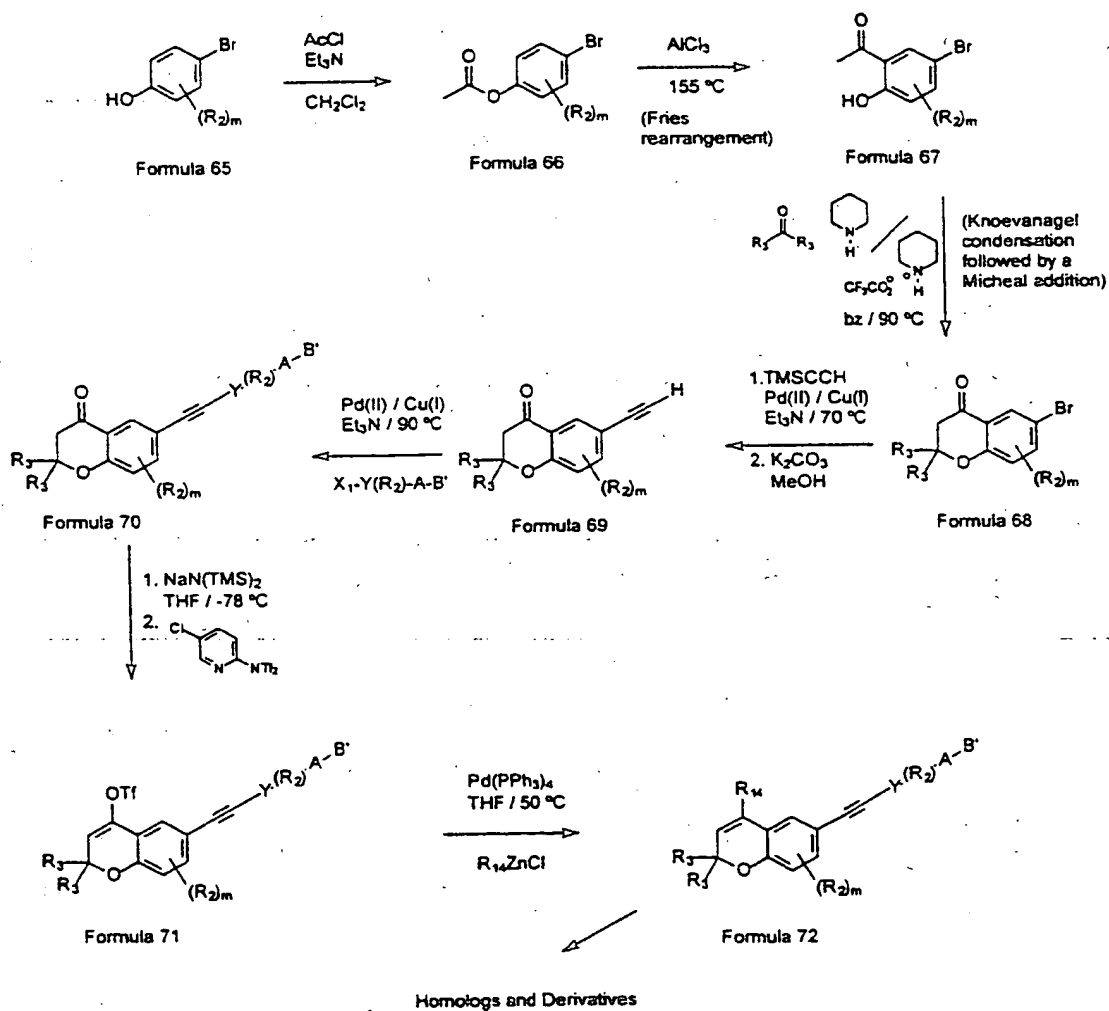
29 2-ethylthiophene, THF, *n*-butyllithium and  $ZnCl_2$  followed by the

1 reaction with the triflate in the presence of  
2 tetrakis(triphenylphosphine)palladium(0);

3 2-*t*-butylthiophene, THF, *n*-butyllithium and ZnCl<sub>2</sub> followed by the  
4 reaction with the triflate in the presence of  
5 tetrakis(triphenylphosphine)palladium(0), and

6 4-bromo-2-methylpyridine, THF, *t*-butyllithium and ZnCl<sub>2</sub> followed by  
7 the reaction with the triflate in the presence of  
8 tetrakis(triphenylphosphine)palladium(0).

9 The 4-aryl or 4-heteroaryl benzothiopyran (thiochromene) derivatives  
10 represented by Formula 64 are preferred compounds within the scope of the  
11 invention. Most preferably the R<sub>3</sub> group is methyl in these compounds. The  
12 compounds of Formula 64 can be converted to further homologs and  
13 derivatives as described above in connection with the preceding reaction  
14 schemes. A frequently utilized reaction in this regard, which gives rise to a  
15 number of specific exemplary compounds of the invention is saponification of  
16 an alkyl ester (B or B' represents an esterified carboxylic acid group) to yield  
17 a free carboxylic acid or its pharmaceutically acceptable salt.





1       A number of particularly preferred compounds of the invention, shown  
2   in Formula 5b and Table 1a, where with reference to Formula 1 X is O and  
3   the 2-position of the benzopyrane (chromene) ring of the compounds is  
4   substituted with alkyl groups, are prepared in accordance with Reaction  
5   Scheme 10. A 4-bromophenol compound of Formula 65 serves as a starting  
6   material and  $R_2$  is defined as in connection with Formula 1, although  
7   preferably the 4 bromophenol is unsubstituted, or is alkyl, halogen or alkoxy  
8   substituted (the  $R_2$  group symbolizes alkyl, halogen or alkoxy). The 4-  
9   bromophenol of Formula 65 is acetylated to provide the acetyloxy-4-  
10   bromobenzene derivative of Formula 66. The acetyloxy-4-bromobenzene  
11   derivative of Formula 66 is then subjected to a rearrangement reaction (*Fries*  
12   rearrangement) by heating with aluminum chloride ( $AlCl_3$ ) to provide the 3-  
13   bromo-6-hydroxyacetophenone derivatives of Formula 67. The latter  
14   compounds are heated in piperidine in the presence of piperidine  
15   trifluoroacetate with a ketone of the formula  $R_3-CO-R_3$ , where the  $R_3$  group  
16   is lower alkyl, causing the 3-bromo-6-hydroxyacetophenone derivatives of  
17   Formula 67 to undergo a *Knoevenagel* condensation followed by a *Michael*  
18   addition, and thereby ring close and provide the 2,2-dialkyl-6-bromo-  
19   chroman-4-one derivatives of Formula 68.

20       The 2,2-dialkyl-6-bromo-chroman-4-one derivatives of Formula 68 are  
21   then reacted with (trimethylsilyl)acetylene under conditions similar to those  
22   described for the reaction with (trimethylsilyl)acetylene of the bromo  
23   compounds of Formula 15 and 20 in Reaction Schemes 1 and 2, respectively,  
24   and for the 6-trifluorosulfonyloxythiochroman-4-one compounds of Formula  
25   60 in Reaction Scheme 9. The resulting 2,2-disubstituted 6-  
26   (trimethylsilyl)ethynyl-chroman-4-one compounds (not shown in Reaction  
27   Scheme 10) are then treated with base (as in the previously described  
28   analogous reactions) to provide 2,2-disubstituted 6-ethynyl-chroman-4-one  
29   compounds of Formula 69. The compounds of Formula 69 are then coupled

1 with the aromatic or heteroaromatic reagent  $X_1-Y(R_2)-A-B'$  (Formula 10,  
2 defined above) under conditions similar to those described for the analogous  
3 reactions of Reaction Scheme 1, Reaction Scheme 2 and Reaction Scheme 9  
4 to yield the compounds of Formula 70. Examples for the reagent  $X_1-Y(R_2)-$   
5  $A-B'$  (Formula 10) used in the synthesis of the herein described specific  
6 preferred embodiments of the compounds of Formula 70 are ethyl 4-  
7 iodobenzoate and ethyl 2-fluoro-4-iodobenzoate.

8 Referring still to Reaction Scheme 10, the 6-(aryl) or  
9 6(heteroaryl)ethynyl 2,2-disubstituted chroman-4-one compounds of Formula  
10 70 are converted into the 6-(aryl) or 6-(heteroaryl)ethynyl 2,2-disubstituted 4-  
11 trifluoromethylsulfonyloxy benzopyran (chromene) derivatives of Formula 71  
12 by treatment with sodium bis(trimethylsilyl)amide and 2-[*N,N*-  
13 bis(trifluoromethylsulfonyl)amino]-5-chloropyridine. This is in analogy to the  
14 conversion of the corresponding thiochroman-4-one compounds (Formula 62)  
15 into the corresponding 4-trifluoromethylsulfonyloxy benzothiopyran  
16 (thiochromene) derivatives of Formula 63. The 6-(aryl) or 6-  
17 (heteroaryl)ethynyl 2,2-disubstituted 4-trifluoromethylsulfonyloxy benzopyran  
18 (chromene) derivatives of Formula 71 are then reacted with an organometal  
19 derivative derived from the aryl or heteroaryl compound  $R_{14}H$ ,  $R_{14}Br$ , or  $R_{14}I$   
20 as described above in connection with Reaction Scheme 1, Reaction Scheme  
21 2 and Reaction Scheme 9, to yield preferred compounds of the invention  
22 shown in Formula 72. Examples of reagents and conditions utilized in  
23 accordance with Reaction Scheme 10 to convert the 4-  
24 trifluoromethanesulfonyloxy compounds of Formula 71 into preferred  
25 compounds of the invention in accordance with Formula 72 are:

26 bromobenzene, THF, *t*-butyllithium and  $ZnCl_2$  followed by the reaction  
27 with the triflate in the presence of tetrakis(triphenylphosphine)palladium(0);  
28 4-methylbromobenzene, THF, *t*-butyllithium and  $ZnCl_2$  followed by the  
29 reaction with the triflate in the presence of

1 tetrakis(triphenylphosphine)palladium(0);

2 4-ethylbromobenzene, THF, *t*-butyllithium and ZnCl<sub>2</sub> followed by the  
3 reaction with the triflate in the presence of

4 tetrakis(triphenylphosphine)palladium(0);

5 4-*iso*-propylbromobenzene, THF, *t*-butyllithium and ZnCl<sub>2</sub> followed by  
6 the reaction with the triflate in the presence of

7 tetrakis(triphenylphosphine)palladium(0), and

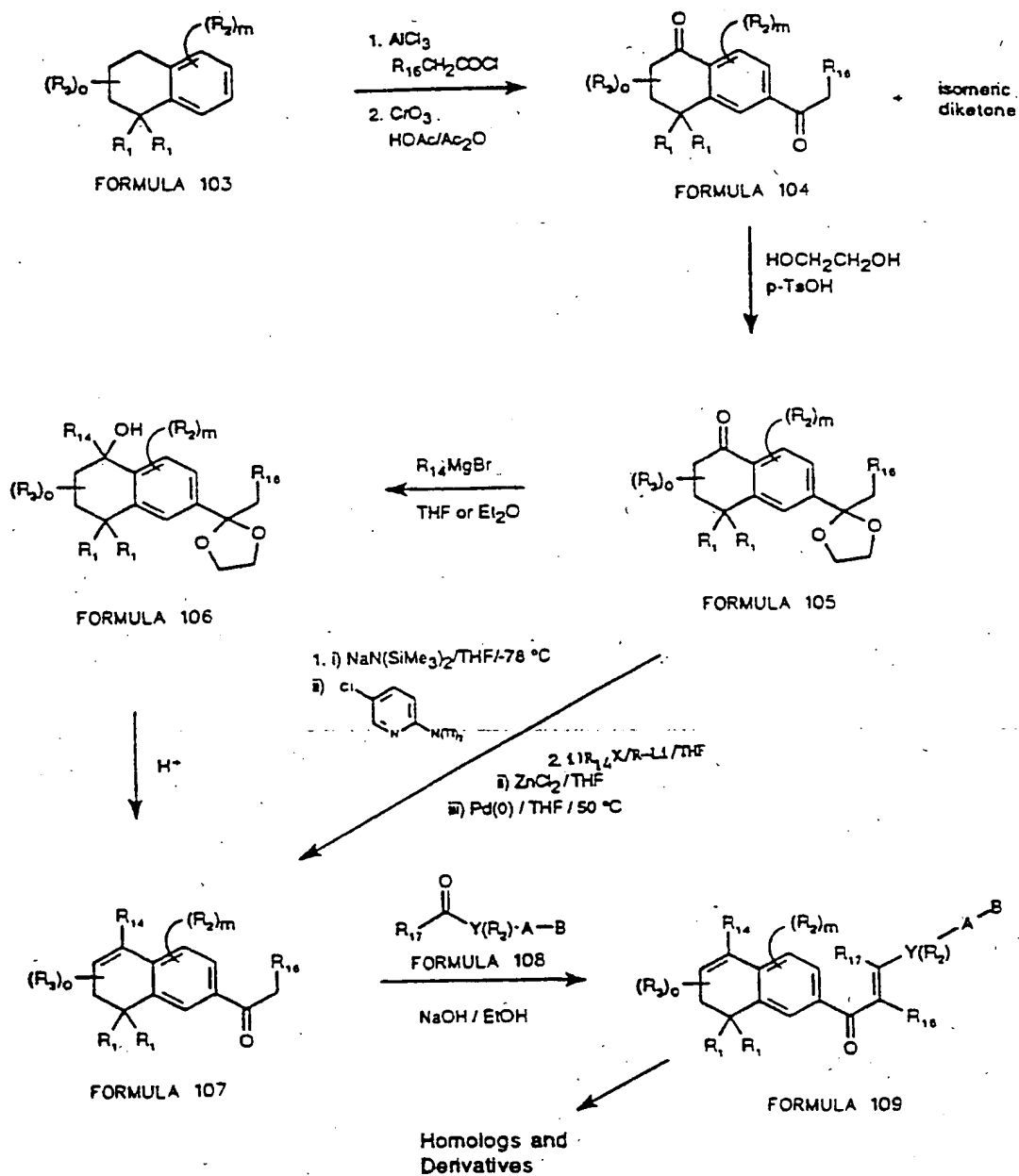
8 4-*t*-butylbromobenzene, THF, *t*-butyllithium and ZnCl<sub>2</sub> followed by the  
9 reaction with the triflate in the presence of

10 tetrakis(triphenylphosphine)palladium(0).

11 The preferred 2,2-disubstituted benzopyran (chromene) compounds of  
12 the invention, represented by Formula 72, can be converted to further  
13 homologs and derivatives, as described above. Saponification of the alkyl  
14 esters represented by Formula 72 when B' represents an esterified carboxyl  
15 group, yields the specially preferred carboxylic acid (or pharmaceutically  
16 acceptable salt) compounds of the invention.

17 Synthetic Methods - Aryl and (3-Oxy-1-Propenyl)-Substituted Compounds

18 The exemplary RAR antagonist compounds of Formula 101 can be  
19 made by the synthetic chemical pathways illustrated here. The synthetic  
20 chemist will readily appreciate that the conditions set out here are specific  
21 embodiments which can be generalized to any and all of the compounds  
22 represented by Formula 101.



1        Reaction Scheme 101 illustrates the synthesis of compounds of  
2        Formula 101 where X is  $[C(R_1)_2]_n$ , n is 1, p is zero and  $R_{17}$  is H or lower  
3        alkyl. In other words, Reaction Scheme 101 illustrates the synthesis of  
4        compounds of the invention which are 3,4-dihydronaphthalene derivatives. In  
5        accordance with this scheme, a tetrahydronaphthalene compound of Formula  
6        103 which is appropriately substituted with the  $R_3$  and  $R_2$  groups (as these  
7        are defined in connection with Formula 101) serves as the starting material.  
8        A preferred example of a compound of Formula 103 is 1,3,3,4-  
9        tetrahydro-1,1-dimethyl-naphthalene, which is described in the chemical  
10       literature (Mathur et al. *Tetrahedron*, 1985, 41:1509. A presently preferred  
11       route for the synthesis of this compound from 1-bromo-3-phenylpropane is  
12       also described in the experimental section of the present application.

13       The compound of Formula 103 is reacted in a Friedel Crafts type  
14       reaction with an acid chloride having the structure  $R_{16}CH_2COCl$  ( $R_{16}$  is  
15       defined as in connection with Formula 101) and is thereafter oxidized with  
16       chromium trioxide in acetic acid to provide the isomeric 6 and 7 acyl-3,4-  
17       dihydro-1(2H)-naphthalenone derivatives. Only the 6-acyl derivative which is  
18       of interest from the standpoint of the present invention, is shown by  
19       structural formula (Formula 104) in Reaction Scheme 101. In the  
20       preparation of the presently preferred compounds of this invention the  $R_1$   
21       groups represent methyl,  $R_2$ ,  $R_3$  and  $R_{16}$  are H, and therefore the preferred  
22       intermediate corresponding to Formula 104 is 3,4-dihydro-4,4-dimethyl-6-  
23       acetyl-1(2H)-naphthalenone.

24       The exocyclic ketone function of the compound of Formula 104 is  
25       thereafter protected as a ketal, for example by treatment with ethylene glycol  
26       in acid, to provide the 1,3-dioxolanyl derivative of Formula 105. The  
27       compound of Formula 105 is then reacted with a Grignard reagent of the  
28       formula  $R_{14}MgBr$  ( $R_{14}$  is defined as in connection with Formula 101) to give  
29       the 1,2,3,4-tetrahydro-1-hydroxy-naphthalene derivative of Formula 106. The

1 exocyclic ketone function of the compound of Formula 106 is then  
2 deprotected by treatment with acid and dehydrated to give the compound of  
3 Formula 107.

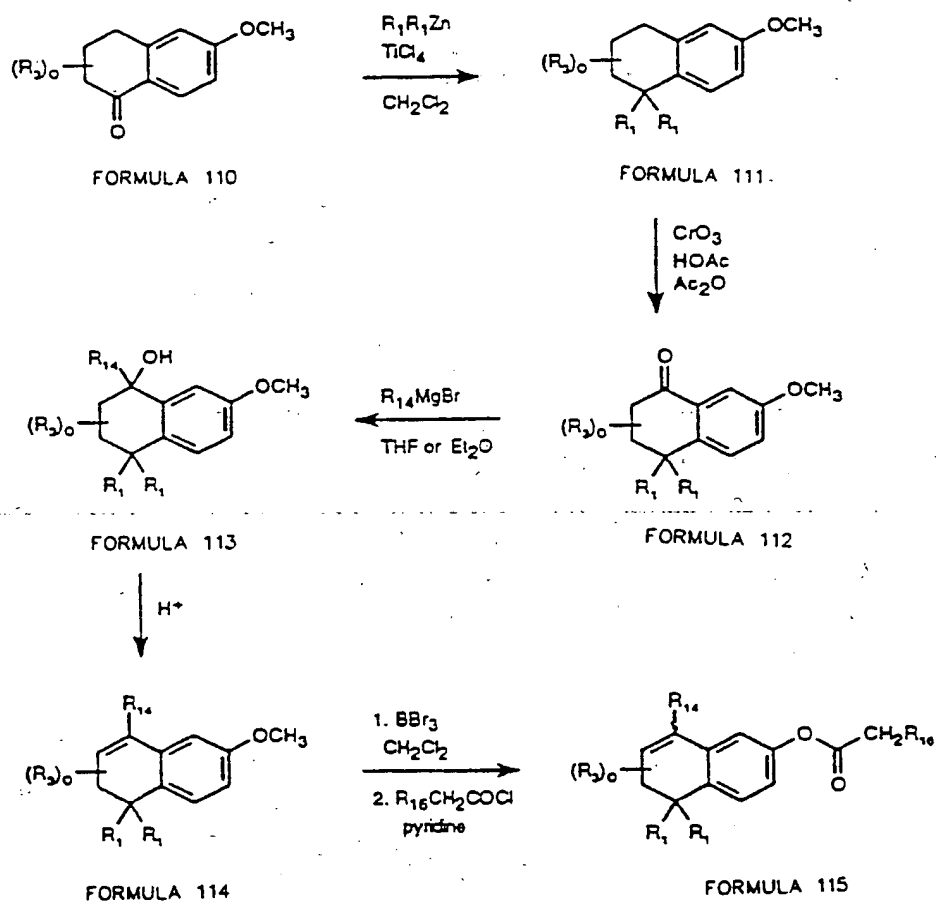
4 An alternate method for obtaining the compounds of Formula 107  
5 from the compounds of Formula 105 is by reacting the compounds of  
6 Formula 105 with sodium bis(trimethylsilyl)amide and 2-[N,N-  
7 bis(trifluoromethylsulfonyl)amino]-5-chloropyridine (Tf =  $\text{SO}_2\text{CF}_3$ ) in an inert  
8 ether type solvent, such as tetrahydrofuran, at low temperatures ( $-78^\circ\text{C}$  and  
9  $0^\circ\text{C}$ ). This reaction proceeds through a sodium salt intermediate which is  
10 usually not isolated and is not shown in Reaction Scheme 101. The overall  
11 reaction results in a trifluoromethylsulfonyloxy derivative, which is thereafter  
12 reacted with an organometal derivative derived from the aryl or heteroaryl  
13 compound  $\text{R}_{14}\text{H}$ , such that the formula of the organometal derivative is  
14  $\text{R}_{14}\text{Met}$  (Met stands for monovalent metal), preferably  $\text{R}_{14}\text{Li}$ . ( $\text{R}_{14}$  is defined  
15 as in connection with Formula 101.) The reaction with the organometal  
16 derivative, preferably lithium derivative of the formula  $\text{R}_{14}\text{Li}$  is usually  
17 conducted in an inert ether type solvent (such as tetrahydrofuran) in the  
18 presence of zinc chloride ( $\text{ZnCl}_2$ ) and tetrakis(triphenylphosphine)-  
19 palladium(0) ( $\text{Pd}(\text{PPh}_3)_4$ ). The organolithium reagent  $\text{R}_{14}\text{Li}$ , if not  
20 commercially available, can be prepared from the compound  $\text{R}_{14}\text{H}$  (or its  
21 halogen derivative  $\text{R}_{14}\text{-X}_1$  where  $\text{X}_1$  is halogen) in an ether type solvent in  
22 accordance with known practice in the art. The temperature range for the  
23 reaction between the reagent  $\text{R}_{14}\text{Li}$  and the trifluoromethylsulfonyloxy  
24 derivative is, generally speaking, in the range of approximately  $-78^\circ\text{C}$  to  $50^\circ\text{C}$ .

25 The compounds of the invention are formed as a result of a  
26 condensation between the ketone compound of Formula 107 and an  
27 aldehyde or ketone of Formula 108. In the preparation of the preferred  
28 exemplary compounds of the invention the reagent of Formula 108 is 4-  
29 carboxybenzaldehyde ( $\text{R}_{17}\text{-H}$ ). Examples of other reagents within the scope

1 of Formula 108 and suitable for the condensation reaction and for the  
2 synthesis of compounds within the scope of the present invention (Formula  
3 101) are: 5-carboxy-pyridine-2-aldehyde, 4- carboxy-pyridine-2-aldehyde, 4-  
4 carboxy-thiophene-2- aldehyde, 5-carboxy-thiophene-2-aldehyde, 4-carboxy-  
5 furan-2-aldehyde, 5-carboxy-furan-2-aldehyde, 4- carboxyacetophenone, 2-  
6 acetyl-pyridine-5-carboxylic acid, 2-acetyl-pyridine-4-carboxylic acid, 2-acetyl-  
7 thiophene-4-carboxylic acid, 2-acetyl-thiophene-5- carboxylic acid, 2-acetyl-  
8 furan-4-carboxylic acid, and 2-acetyl-furan-5-carboxylic acid. The latter  
9 compounds are available in accordance with the chemical literature; see for  
10 example Decroix et al., *J. Chem. Res.(S)*, 4: 134 (1978); Dawson et al., *J. Med.*  
11 *Chem.* 29:1282 (1983); and Queguiner et al., *Bull Soc. Chimique de France*  
12 No. 10, pp. 3678 - 3683 (1969). The condensation reaction between the  
13 compounds of Formula 107 and Formula 108 is conducted in the presence of  
14 base in an alcoholic solvent. Preferably, the reaction is conducted in ethanol  
15 in the presence of sodium hydroxide. Those skilled in the art will recognize  
16 this condensation reaction as an aldol condensation, and in case of the herein  
17 described preferred examples (condensing a ketone of Formula 107 with an  
18 aldehyde of Formula 108) as a Claisen-Schmidt reaction. (See March:  
19 Advanced Organic Chemistry: Reactions, Mechanisms, and Structure, pp. 694  
20 - 695 McGraw Hill (1968). The compounds of Formula 109 are within the  
21 scope of the present invention, and can also be subjected to further  
22 transformations resulting in additional compounds of the invention.  
23 Alternatively, the A-B group of Formula 108 may be a group which is within  
24 the scope of the invention, as defined in Formula 101, only after one or more  
25 synthetic transformations of such a nature which is well known and within the  
26 skill of the practicing organic chemist. For example, the reaction performed  
27 on the A-B group may be a deprotection step, homologation, esterification,  
28 saponification, amide formation or the like.

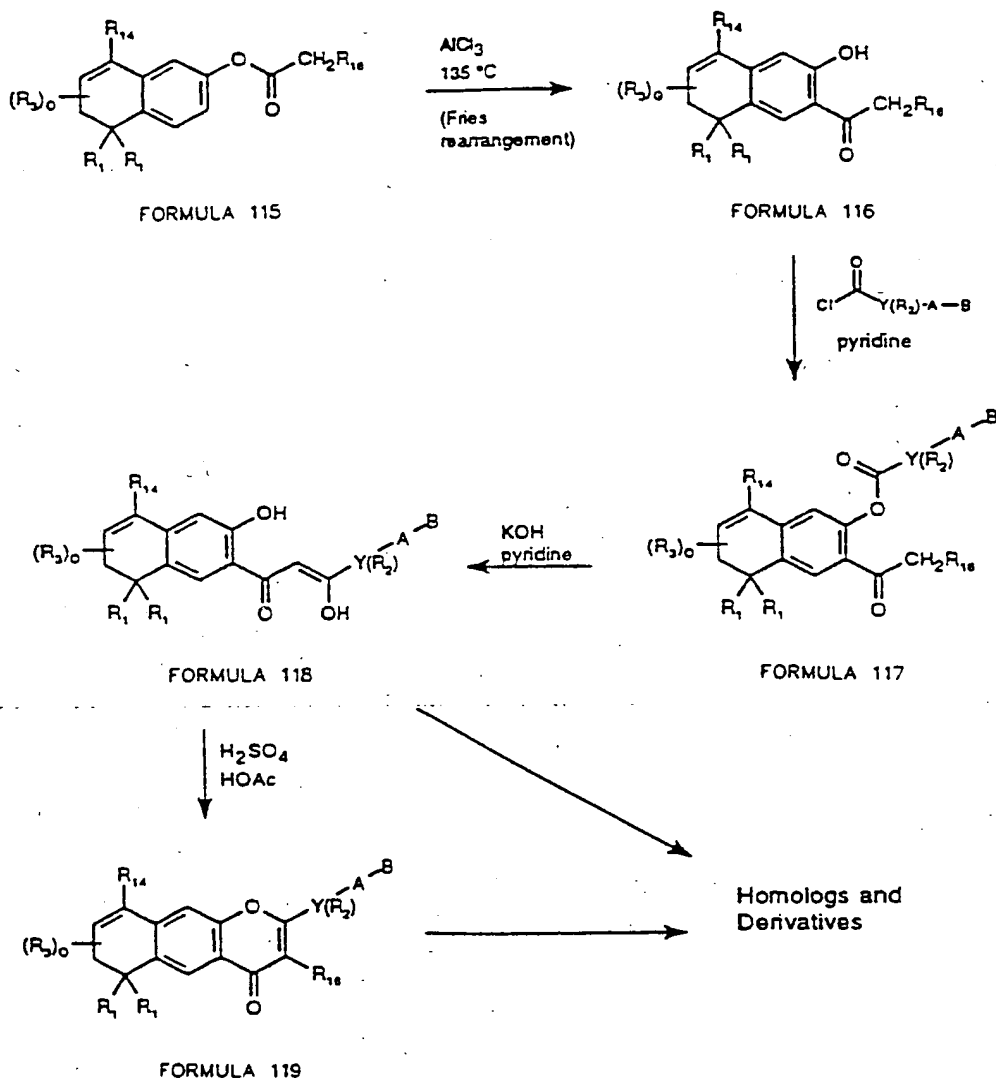
29 Generally speaking, regarding derivatization of compounds of Formula

1 109 and/or the synthesis of aryl and heteroaryl compounds of Formula 108  
2 which can thereafter be reacted with compounds of Formula 107, well known  
3 and published general principles and synthetic methodology can be employed  
4 which is also described above under the subtitle "Synthetic Methods - Aryl  
5 Substituted Compounds".



Reaction Scheme 102





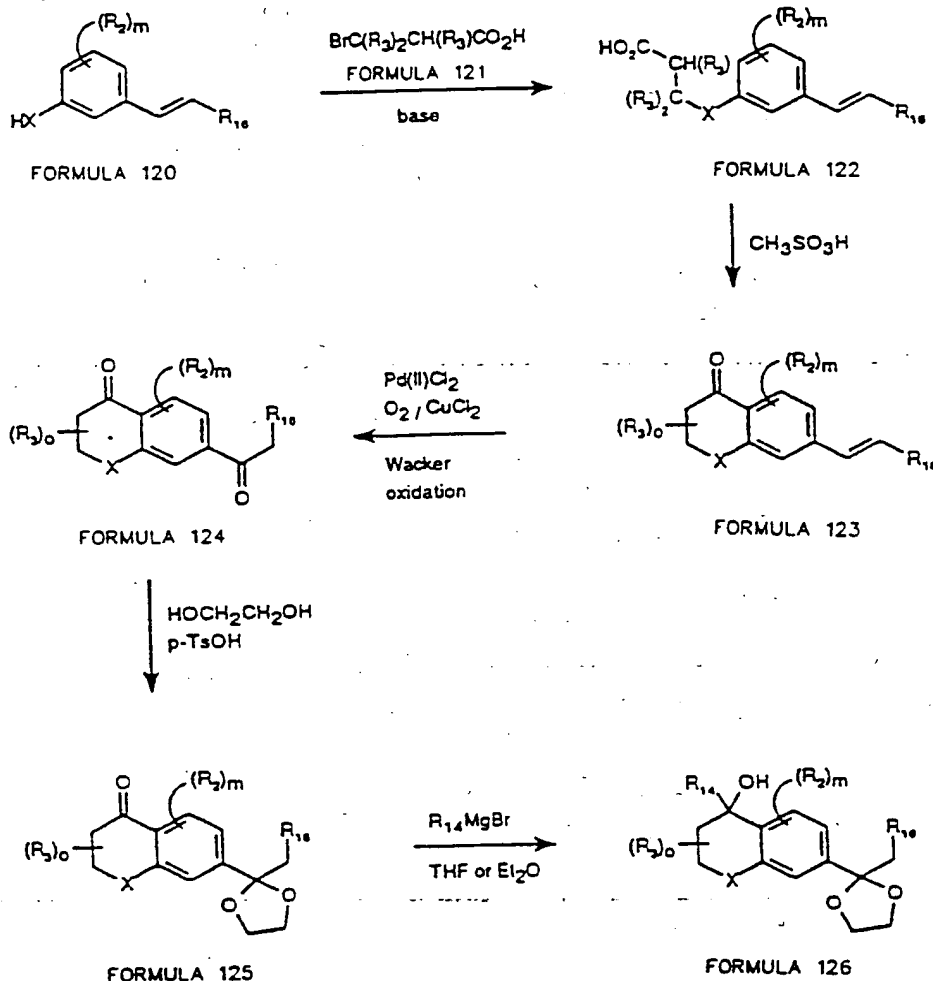
1 Referring now to Reaction Scheme 102, a synthetic route to those  
2 compounds of the invention is described in which, with reference to Formula  
3 101 p is zero,  $R_2$  in the aromatic portion of the condensed ring structure is  
4 OH and  $R_{17}$  is OH. Those skilled in the art will readily recognize that these  
5 compounds are  $\beta$ -diketones in the enol form. Reaction Scheme 102 also  
6 describes a synthetic route to those compounds of the invention where p is 1.  
7 Those skilled in the art will readily recognize that the latter compounds are  
8 flavones. Thus, in accordance with this scheme a 1,2,3,4- tetrahydro-6-  
9 methoxynaphthalene-1-one derivative of Formula 110 is reacted with dialkyl  
10 zinc ( $R_1Zn$ ) in the presence of titanium tetrachloride in a suitable solvent  
11 such as  $CH_2Cl_2$  to replace the oxo function with the geminal dialkyl group  
12  $R_1R_1$ , to yield a compound of Formula 111, where  $R_1$  is lower alkyl. In  
13 preferred embodiments of the compounds of the invention which are made in  
14 accordance with Reaction Scheme 102 the  $R_3$  group is hydrogen and  $R_1$  are  
15 methyl. Accordingly, the dialkyl zinc reagent is dimethyl zinc, and the  
16 preferred starting material of Formula 110 is 1,2,3,4- tetrahydro-6-  
17 methoxynaphthalene-1-one. The latter compound is commercially available,  
18 for example from Aldrich Chemical Company. The 1,2,3,4-tetrahydro-1,2-  
19 dialkyl-6-methoxy naphthalene derivative of Formula 111 is thereafter  
20 oxidized with chromium trioxide in acetic acid and acetic anhydride to give a  
21 1,2,3,4-tetrahydro- 3,4-dialkyl-7-methoxy naphthalen-1-one derivative of  
22 Formula 112. The ketone compound of Formula 112 is reacted with a  
23 Grignard reagent ( $R_{14}MgBr$ ,  $R_{14}$  is defined as in connection with Formula  
24 101) to yield a 1- hydroxy-1-aryl-3,4-dihydro-3,4-dialkyl-7-methoxy  
25 naphthalene derivative of Formula 113. The hydroxy compound of Formula  
26 113 is subjected to elimination by heating, preferably in acid, to yield the  
27 dihydronaphthalene compound of Formula 114. The methyl group is  
28 removed from the phenolic methyl ether function of the compound of  
29 Formula 114 by treatment with boron tribromide in a suitable solvent, such as

1  $\text{CH}_2\text{Cl}_2$ , and thereafter the phenolic OH is acylated with an acylating agent  
2 that introduces the  $\text{R}_{16}\text{CH}_2\text{CO}$  group, to give a compound of Formula 115.  
3 In the preferred embodiment  $\text{R}_{16}$  is H, and therefore the acylating agent is  
4 acetyl chloride or acetic anhydride. The acetylation reaction is conducted in  
5 a basic solvent, such as pyridine. The acylated phenol compound of Formula  
6 115 is reacted with aluminum chloride at elevated temperature, causing it to  
7 undergo a Fries rearrangement and yield the 1-aryl-3,4-dialkyl-3,4-dihydro-6-  
8 acyl-7-hydroxy-naphthalene compound of Formula 116. The phenolic  
9 hydroxyl group of the compound of Formula 116 is acylated with an  
10 acylating agent (such as an acid chloride) that introduces the  $\text{CO-Y(R}_2\text{)-A-B}$   
11 group to yield a compound of Formula 117. In the acid chloride reagent  $\text{Cl-}$   
12  $\text{CO-Y(R}_2\text{)-A-B}$  (or like acylating reagent) the symbols Y,  $\text{R}_2$  and A-B have  
13 the meaning defined in connection with Formula 101. In the preparation of  
14 a preferred compound of the invention in accordance with this scheme this  
15 reagent is  $\text{ClCOC}_6\text{H}_4\text{COOEt}$  (the half ethyl ester half acid chloride of  
16 terephthalic acid).

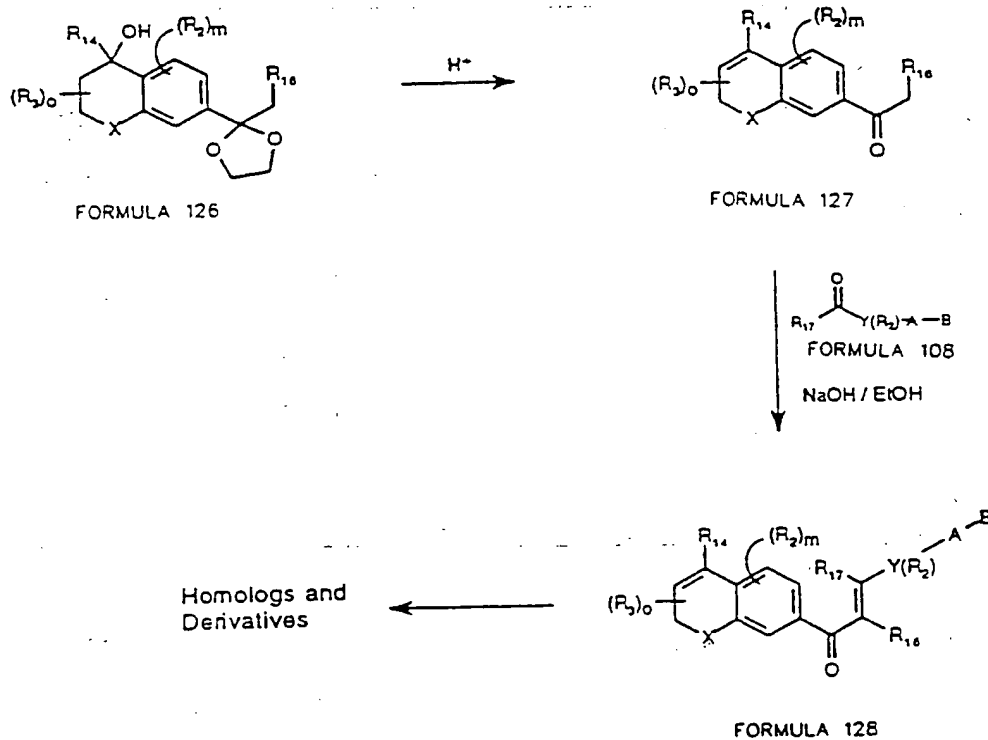
17 The compound of Formula 117 is reacted with strong base, such as  
18 potassium hydroxide in pyridine, to yield, as a result of an intramolecular  
19 Claisen condensation reaction, a compound of Formula 118. The compounds  
20 of Formula 118 are within the scope of the invention and of Formula 101,  
21 where there is an OH for the  $\text{R}_2$  substituent in the aromatic portion of the  
22 condensed ring moiety and  $\text{R}_{17}$  is OH. In connection with the foregoing  
23 reaction (intramolecular Claisen condensation) and the previously mentioned  
24 Fries rearrangement it is noted that these probable reaction mechanisms are  
25 mentioned in this description for the purpose of fully explaining the herein  
26 described reactions, and for facilitating the work of a person of ordinary skill  
27 in the art to perform the herein described reactions and prepare the  
28 compounds of the invention. Nevertheless, the present inventors do not wish  
29 to be bound by reaction mechanisms and theories, and the herein claimed

1 invention should not be limited thereby.

2 The compounds of Formula 118 are reacted with strong acid, such as  
 3 sulfuric acid, in a suitable protonic solvent, such as acetic acid, to yield the  
 4 flavone compounds of Formula 119. The compounds of Formula 119 are  
 5 also compounds of the invention, within the scope of Formula 101 where p is  
 6 1. Both the compounds of Formula 118 and Formula 119 can be subjected  
 7 to further reactions and transformations to provide further homologs and  
 8 derivatives, as described above in connection with Reaction Scheme 101.  
 9 This is indicated in Reaction Scheme 102 as conversion to homologs and  
 10 derivatives.



Reaction Scheme 103



Reaction Scheme 103 (continued)

1 Referring now to Reaction Scheme 103 a synthetic route is shown  
2 leading to those compounds of the invention where, with reference to  
3 Formula 101 X is S, O or NR', p is zero and R<sub>17</sub> is H or lower alkyl.  
4 However, by applying the generic principles of synthesis shown in Reaction  
5 Scheme 102 the presently shown synthetic process can be modified or  
6 adapted by those of ordinary skill in the art to also obtain compounds of the  
7 invention where X is S, O or NR' and p is 1, or where X is S, O or NR' and  
8 p is zero, the R<sub>2</sub> group in the aromatic portion of the condensed ring moiety  
9 is OH and R<sub>17</sub> is OH.

10 The starting compound of Reaction Scheme 103 is a phenol,  
11 thiophenol or aniline derivative of Formula 120. In the presently preferred  
12 compounds of the invention the R<sub>2</sub> and R<sub>16</sub> groups are both hydrogen, and  
13 the preferred starting compounds of Formula 120 are 3-ethenyl-thiophenol  
14 or 3-ethenyl-phenol which are known in the chemical literature (Nuyken, et  
15 al. *Polym. Bull* (Berlin) 11:165 (1984). For the sake of simplifying the present  
16 specification, in the ensuing description X can be considered primarily sulfur  
17 as for the preparation of benzothiopyran derivatives of the present invention.  
18 It should be kept in mind, however, that the herein described scheme is also  
19 suitable, with such modifications which will be readily apparent to those  
20 skilled in the art, for the preparation of benzopyran (X = O) and  
21 dihydroquinoline (X = NR') compounds within the scope of the present  
22 invention. Thus, the compound of Formula 120 is reacted under basic  
23 condition with a 3-bromo carboxylic acid of the Formula 121. In this reaction  
24 scheme the symbols have the meaning described in connection with Formula  
25 101. An example for the reagent of Formula 121 where R<sub>3</sub> is hydrogen, is 3-  
26 bromopropionic acid. The reaction with the 3-bromocarboxylic acid of  
27 Formula 121 results in the compound of Formula 122. The latter is cyclized  
28 by treatment with acid to yield the 7-ethenyl-thiochroman-4-one derivative  
29 (when X is S) or 7-ethenyl-chroman derivative (when X is O) of Formula 123.

1 The 7-ethenyl- thiochroman-4-one or 7-ethenyl-chroman-4-one derivative of  
2 Formula 123 is oxidized in the presence of  $\text{Pd(II)Cl}_2$  and  $\text{CuCl}_2$  catalysts to  
3 provide the corresponding 7-acyl (ketone) compound of Formula 124. Those  
4 skilled in the art will recognize the latter reaction as a Wacker oxidation.  
5 The exocyclic ketone group of the compound of Formula 124 is protected in  
6 the form of a ketal, for example by treatment with ethylene glycol in acid, to  
7 provide the 1,3-dioxolanyl derivative of Formula 125. Thereafter the  
8 compound of Formula 125 is subjected to a sequence of reactions analogous  
9 to those described for the compounds of Formula 105 in Reaction Scheme  
10 101. Thus, the 1,3- dioxolanyl derivative of Formula 125 is reacted with a  
11 Grignard reagent of the formula  $\text{R}_{14}\text{MgBr}$  to give the tertiary alcohol of  
12 Formula 126, which is thereafter dehydrated in acid to provide the  
13 benzothiopyran (X is S), benzopyran (X is O) or dihydroquinoline (X is  $\text{NR}'$ )  
14 derivative of Formula 127. The ketone compound of Formula 127 is then  
15 reacted in the presence of base with the reagent of Formula 108 in an aldol  
16 condensation (Claisen-Schmidt) reaction to provide compounds of the  
17 invention of Formula 128. The compounds of Formula 128 can be converted  
18 into further homologs and derivatives, as described above in connection with  
19 Reaction Schemes 101 and 102.

#### 20 Specific Examples

##### 21 2-hydroxy-2-methyl-5-phenylpentane

22 To a mixture of magnesium turnings 13.16 g (0.541 mol) in 200 ml of  
23 anhydrous  $\text{Et}_2\text{O}$  was added 100.0 g (0.492 mol) of 1-bromo-3-phenyl propane  
24 as a solution in 100 ml of  $\text{Et}_2\text{O}$ . After of 5-10 ml of the solution had been  
25 added, the addition was stopped until the formation of the Grignard reagent  
26 was in progress. The remaining bromide was then added over 1 hour. The  
27 Grignard reagent was stirred for 20 minutes at  $35^\circ\text{C}$  and then 31.64 g (0.541  
28 mol) of acetone was added over a 45 minute period. The reaction was  
29 stirred overnight at room temperature before being cooled to  $0^\circ\text{C}$  and

1 acidified by the careful addition of 20% HCl. The aqueous layer was  
2 extracted with Et<sub>2</sub>O (3 x 200 ml) and the combined organic layers washed  
3 with water, and saturated aqueous NaCl before being dried over MgSO<sub>4</sub>.  
4 Removal of the solvent under reduced pressure and distillation of the residue  
5 afforded 63.0g (72%) of the product as a pale-yellow oil, bp 99-102°C / 0.5  
6 mm Hg. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.28-7.18 (5H, m), 2.63 (2H, t, J = 7.5 Hz),  
7 1.68 (2H, m), 1.52 (2H, m), 1.20 (6H, s).

8 1,2,3,4-tetrahydro-1,1-dimethylnaphthalene

9 A mixture of P<sub>2</sub>O<sub>5</sub> (55.3 g, 0.390 mol) in 400 ml of methanesulfonic  
10 acid was heated to 105°C under argon until all of the solid had dissolved.  
11 The resulting solution was cooled to room temperature and 2-hydroxy-2-  
12 methyl-5-phenylpentane (63.0 g, 0.354 mol) added slowly with stirring. After  
13 4 hours the reaction was quenched by carefully pouring the solution onto 1 L  
14 of ice. The resulting mixture was extracted with Et<sub>2</sub>O (4 x 125 ml) and the  
15 combined organic layers washed with water, saturated aqueous NaHCO<sub>3</sub>,  
16 water, and saturated aqueous NaCl before being dried over MgSO<sub>4</sub>.  
17 Concentration of the solution under reduced pressure, followed by distillation  
18 afforded 51.0 g (90%) of the product as a clear colorless oil, bp. 65-67°C / 1.1  
19 mmHg. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.32 (1H, d, J = 7.4 Hz), 7.16-7.05 (3H, m),  
20 2.77 (2H, t, J = 6.3 Hz), 1.80 (2H, m), 1.66 (2H, m), 1.28 (6H, s).

21 3,4-dihydro-4,4-dimethyl-1(2H)-naphthalenone (Compound A)

22 A solution of 350 ml of glacial acetic acid and 170 ml of acetic  
23 anhydride was cooled to 0°C and CrO<sub>3</sub>, 25.0 g (0.25 mol) carefully added in  
24 small portions. The resulting mixture was stirred for 30 minutes before 120  
25 ml of benzene was added. 1,2,3,4-tetrahydro-1,1-dimethylnaphthalene was  
26 added slowly as a solution in 30 ml of benzene. Upon completing the  
27 addition the reaction was stirred for 4 hours at 0°C. The solution was diluted  
28 with H<sub>2</sub>O (200 ml) and extracted with Et<sub>2</sub>O (5 x 50 ml). The combined  
29 organic layers were washed with water, saturated aqueous NaCO<sub>3</sub>, and



1 saturated aqueous NaCl, before being dried over  $\text{MgSO}_4$ . Removal of the  
2 solvents under reduced pressure, and distillation afforded 16.0 g (74%) of the  
3 product as a pale-yellow oil, bp  $93-96^\circ\text{C}$  / 0.3 mm Hg  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$   
4 8.02 (1H, dd,  $J = 1.3, 7.8$  Hz), 7.53 (1H, m), 7.42 (1H, d,  $J = 7.9$  Hz), 7.29  
5 (1H, m), 2.74 (2H, t,  $J = 6.8$  Hz), 2.02 (2H, t,  $J = 6.8$  Hz), 1.40 (6H, s).  
6 3,4-dihydro-4,4-dimethyl-7-bromo-1(2H)-naphthalenone (Compound B)

7 A 100 ml three-necked flask, fitted with an efficient reflux condenser  
8 and drying tube, and addition funnel, was charged with a mixture of  $\text{AlCl}_3$   
9 9.5g (71.4 mmol) and 3 ml of  $\text{CH}_2\text{Cl}_2$ . The 3,4-dihydro- 4,4-dimethyl-1(2H)-  
10 naphthalenone (5.0g, 28.7 mmol), was added dropwise with stirring (Caution:  
11 Exothermic Reaction!) to the mixture at room temperature. Bromine, 5.5 g  
12 (34.5 mmol), was then added very slowly, and the resulting mixture stirred for  
13 2 hours at room temperature. (Note: if stirring stops, the mixture can be  
14 warmed to  $70^\circ\text{C}$  until stirring resumes.) The reaction was then quenched by  
15 the slow addition of ice-cold 6M HCl. The mixture was extracted with  $\text{Et}_2\text{O}$   
16 and the combined organic layers washed with water, saturated aqueous  
17  $\text{NaHCO}_3$ , and saturated NaCl, before being dried over  $\text{MgSO}_4$ . Removal of  
18 the solvent under reduced pressure, and distillation of the residue afforded  
19 5.8 g (80%) of the product as a pale-yellow oil which solidified on standing,  
20 bp:  $140^\circ\text{C}$  / 0.4 mm Hg.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  8.11 (1H, d,  $J = 3.0$  Hz), 7.61  
21 (1H, dd,  $J = 3.0, 9.0$  Hz), 7.31 (1H, d,  $J = 9.0$  Hz), 2.72 (2H, t,  $J = 6.0$  Hz),  
22 2.01 (2H, t,  $J = 6.0$  Hz), 1.28 (6H, s).

23 1,2,3,4-tetrahydro-1-hydroxy-1-(4-methylphenyl)-4,4-dimethyl-7-  
24 bromonaphthalene (Compound C)

25 To a mixture of magnesium turnings (648.0 mg, 27.0 mmol) in 25 ml of  
26 THF was added a solution of 4-bromotoluene (5.40 g, 31.8 mmol) in 10 ml  
27 of THF in two portions. The reaction was initiated by the addition of 2 ml of  
28 the solution, followed by the slow addition of the remaining solution via an  
29 addition funnel. The mixture was stirred at room temperature for 1 hour,

1 and then the solution was transferred to a second flask using a canula. To  
2 the resulting Grignard reagent was added 4.0 g (15.9 mmol) of 3,4-dihydro-  
3 4,4-dimethyl-7-bromo-1(2H)-naphthalenone (Compound B) as a solution in  
4 15 ml of THF. The resulting solution was heated to reflux overnight, cooled  
5 to room temperature, and the reaction quenched by the careful addition of  
6 ice-cold 10% HCl. Extraction with Et<sub>2</sub>O was followed by washing of the  
7 combined organic layers with H<sub>2</sub>O and saturated aqueous NaCl, then drying  
8 over MgSO<sub>4</sub>. Removal of the solvent under reduced pressure provided an oil  
9 which afforded the product as a colorless solid after column chromatography  
10 (hexanes / EtOAc, 96 : 4). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.36 (1H, dd, J = 2.1, 7.6  
11 Hz), 7.26 (3H, m), 7.12 (3H, s), 2.34 (3H, s), 2.24-2.04 (2H, m), 1.81 (1H, m),  
12 1.55 (1H, m), 1.35 (3H, s), 1.30 (3H, s).

13 3,4-dihydro-1-(4-methylphenyl)-4,4-dimethyl-7-bromonaphthalene  
14 (Compound D)

15 A flask equipped with a Dean-Stark trap was charged with 3.4 g of  
16 (9.85 mmol) of 1,2,3,4-tetrahydro-1-hydroxy-1-(4-methylphenyl)-4,4-  
17 dimethyl-7-bromonaphthalene (Compound C) and 40 ml of benzene. A  
18 catalytic amount of p-toluenesulfonic acid monohydrate was added and the  
19 resulting solution heated to reflux for 2 hours. Upon cooling to room  
20 temperature, Et<sub>2</sub>O was added and the solution washed with H<sub>2</sub>O, saturated  
21 aqueous NaHCO<sub>3</sub>, and saturated aqueous NaCl then dried over MgSO<sub>4</sub>.  
22 Removal of the solvents under reduced pressure, and column  
23 chromatography (100% hexane / silica gel) afforded the title compound as a  
24 colorless solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.32 (1H, dd, J = 2.1, 8.2 Hz), 7.21  
25 (5H, m), 7.15 (1H, d, J = 2.1 Hz), 5.98 (1H, t, J = 4.7 Hz), 2.40 (3H, s), 2.32  
26 (2H, d, J = 4.7 Hz), 1.30 (6H, s).

27 7-Ethynyl-3,4-dihydro-4,4-dimethylnaphthalen-1(2H)-one (Compound E)

28 To a solution (flushed for 15 minutes with a stream of argon) of 7 g  
29 (27.6 mmol) of 3,4-dihydro-4,4-dimethyl-7-bromo-1(2H)-naphthalenone

1 (Compound B) in 150 ml of triethylamine was added 0.97 g (1.3 mmol) of  
2 bis(triphenylphosphine)palladium(II) chloride and 0.26 g (1.3 mmol) of  
3 cuprous iodide. The solution mixture was flushed with argon for 5 minutes  
4 and then 39 ml (36.6 mmol) of (trimethylsilyl)acetylene was added. The  
5 reaction mixture was sealed in a pressure tube and placed in a preheated oil  
6 bath (100°C) for 24 hours. The reaction mixture was then filtered through  
7 Celite, washed with Et<sub>2</sub>O and the filtrate concentrated in vacuo to give crude  
8 7-(trimethylsilyl)ethynyl-3,4-dihydro-4,4-dimethylnaphthalen-1(2H)-one. To a  
9 solution of this crude TMS-acetylenic compound in 50 ml of methanol was  
10 added 0.6 g (4.3 mmol) of K<sub>2</sub>CO<sub>3</sub>. The mixture was stirred for 8 hours at  
11 ambient temperature and then filtered. The filtrate was concentrated in  
12 vacuo, diluted with Et<sub>2</sub>O, washed with water, 10% HCl and brine, dried over  
13 MgSO<sub>4</sub> and concentrated in vacuo. Purification by column chromatography  
14 (silica, 10% EtOAc-hexane) yielded the title compound as a white solid.  
15 PMR (CDCl<sub>3</sub>) : δ 1.39 (6H, s), 2.02 (2H, t, J = 7.0 Hz), 2.73 (2H, t, J = 7.0  
16 Hz), 3.08 (1H, s), 7.39 (1H, d, J = 8.2 Hz), 7.61 (1H, dd, J = 1.8, 8.2 Hz),  
17 8.14 (1H, d, J = 9.18 Hz).

18 Ethyl-4-iodobenzoate

19 To a suspension of 10 g (40.32 mmol) of 4-iodobenzoic acid in 100 ml  
20 absolute ethanol was added 2 ml thionyl chloride and the mixture was then  
21 heated at reflux for 3 hours. Solvent was removed in vacuo and the residue  
22 was dissolved in 100 ml ether. The ether solution was washed with saturated  
23 NaHCO<sub>3</sub> and saturated NaCl solutions and dried (MgSO<sub>4</sub>). Solvent was then  
24 removed in vacuo and the residue Kugelrohr distilled (100°C; 0.55 mm) to  
25 give the title compound as a colorless oil, PMR (CDCl<sub>3</sub>): δ 1.42 (3H, t, J ~ 7  
26 Hz), 4.4 (2H, q, J ~ 7 Hz), 7.8 (4H).

27 6-iodonicotinic acid

28 Sodium iodide (20.59 g, 137.40 mmol) was cooled to -78°C under argon  
29 and then hydriodic acid (97.13 g, 759.34 mmol) was added. The cooling bath

1 was removed and the suspension was stirred for 5 minutes. To this mixture  
2 was added 6-chloronicotinic acid (22.09 g, 140.20 mmol) and the resulting  
3 mixture was slowly warmed to ambient temperature with stirring. The  
4 mixture was heated to reflux at 125°C for 24 hours, cooled to ambient  
5 temperature and poured into acetone (500 ml) at 0°C. The yellow solid was  
6 collected by filtration and washed with 200 ml of 1N aqueous NaHSO<sub>3</sub>  
7 solution. Recrystallization from methanol (crystals were washed with ethyl  
8 ether) afforded the title compound as white crystals: mp 177-179°C [lit. mp  
9 187-192, Newkome et al. "Reductive Dehalogenation of Electron-Poor  
10 Heterocycles: Nicotinic Acid Derivatives" *J. Org. Chem.* 51: 953-954 (1986).  
11 <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 8.81 (1H, dd, J = 0.8, 2.4 Hz), 8.01 (1H, dd, J =  
12 0.8, 8.2 Hz), 7.91 (1H, dd, J = 2.4, 8.2 Hz).

13 Ethyl 6-iodonicotinoate

14 To a suspension of 6-iodonicotinic acid (23.38 g, 94.20 mmol) in  
15 dichloromethane (100 ml) was added a solution of 1-(3-  
16 dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (19.86 g, 103.6  
17 mmol) in dichloromethane (250 ml). To this mixture was added ethanol  
18 (12.40 g, 269.27 mmol) followed by dimethylaminopyridine (1.15 g, 9.41  
19 mmol). The mixture was heated at 50°C for 24.5 hours, concentrated in  
20 vacuo, and diluted with water (200 ml) then extracted with ethyl ether (550  
21 ml). The combined organic phases were washed with saturated aqueous  
22 NaCl, dried (MgSO<sub>4</sub>) and concentrated to a yellow solid. Purification by  
23 flash chromatography (silica, 10% EtOAc-hexane) afforded the title  
24 compound as white needles: mp 48-49°C; <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 8.94 (1H, d,  
25 J = 2.1 Hz), 7.91 (1H, dd, J = 2.1, 8.2 Hz), 7.85 (1H, d, J = 8.2 Hz), 4.41  
26 (2H, q, J = 7.1 Hz), 1.41 (3H, t, J = 7.1 Hz).

27 Ethyl 4-[(5,6,7,8-tetrahydro-5,5-dimethyl-8-oxo-2-  
28 naphthalenyl)ethynyl]benzoate (Compound F)

29 To a solution of 4 g (21.7 mmol) of 7-ethynyl-3,4-dihydro-4,4-

1 dimethylnaphthalen-1(2H)-one (Compound E ) flushed for 15 minutes with a  
2 stream of argon, and 6 g (21.7 mmol) of ethyl 4-iodobenzoate in 100 ml of  
3 triethylamine was added 5 g (7.2 mmol) of  
4 bis(triphenylphosphine)palladium(II) chloride and 1.4 g (7.2 mmol) of  
5 cuprous iodide. The mixture was flushed with argon for 5 minutes and then  
6 stirred at ambient temperature for 18 hours. The reaction mixture was  
7 filtered through Celite and the filtrate was concentrated in vacuo. Purification  
8 by flash chromatography (silica, 10 % EtOAc-hexane) yielded the title  
9 compound as a white solid. PMR (CDCl<sub>3</sub>) :  $\delta$  1.41 (3H, t, J = 7.2 Hz), 1.41  
10 (6H, s), 2.04 (2H, t, J = 6.5 Hz), 2.76 (2H, t, J = 6.5 Hz), 4.40 (2H, q, J =  
11 7.2 Hz), 7.44 (1H, d, J = 8.2 Hz), 7.59 (2H, d, J = 8.4 Hz), 7.68 (1H, dd, J =  
12 1.8, 8.2 Hz), 8.04 (2H, d, J = 8.4 Hz), 8.15 (1H, d, J = 1.8 Hz).

13 Ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(trifluoromethylsulfonyl)oxy-2-  
14 naphthalenyl)ethynyl]benzoate (Compound G)

15 To a cold solution (-78°C) of 291.6 mg (1.59 mmol) of sodium  
16 bis(trimethylsilyl)amide in 5.6 ml of THF was added a solution of 500.0 mg  
17 (1.44 mmol) of ethyl 4-[(5,6,7,8-tetrahydro-5,5-dimethyl-8-oxo-2-  
18 naphthalenyl)ethynyl]benzoate (Compound F) in 4.0 ml of THF. The  
19 reaction mixture was stirred at -78°C for 35 minutes and then a solution of  
20 601.2 mg (1.59 mmol) of 5-chloro(2-bis-trifluoromethylsulfonyl)imide in 4.0  
21 ml of THF was added. After stirring at -78°C for 1 hour, the solution was  
22 warmed to 0°C and stirred for 2 hours. The reaction was quenched by the  
23 addition of saturated aqueous NH<sub>4</sub>Cl. The mixture was extracted with  
24 EtOAc (50 ml) and the combined organic layers were washed with 5%  
25 aqueous NaOH, water, and brine. The organic phase was dried over Na<sub>2</sub>SO<sub>4</sub>  
26 and then concentrated in vacuo to a yellow oil. Purification by column  
27 chromatography (silica, 7% EtOAc-hexanes) yielded the title compound as a  
28 colorless solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  8.04 (2H, dd, J = 1.8, 8.4 Hz), 7.60  
29 (2H, dd, J = 1.8, 8.4 Hz), 7.51 (2H, m), 7.32 (1H, d, J = 8.0 Hz), 4.40 (2H,

1 q, J = 7.1 Hz), 6.02 (1H, t, J = 5.0 Hz), 2.44 (2H, d, J = 5.0 Hz), 1.43 (3H,  
2 t, J = 7.1 Hz), 1.33 (6H, s).

3 Ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-  
4 naphthalenyl)ethynyl]benzoate (Compound 1)

5 A solution of 4-lithiotoluene was prepared by the addition of 189.9 mg  
6 (1.74 ml, 2.96 mmol) of t-butyl lithium (1.7M solution in hexanes) to a cold  
7 solution (-78°C) of 253.6 mg (1.482 mmol) of 4-bromotoluene in 2.0 ml of  
8 THF. After stirring for 30 minutes a solution of 269.4 mg (1.977 mmol) of  
9 zinc chloride in 3.0 ml of THF was added. The resulting solution was  
10 warmed to room temperature, stirred for 30 minutes, and added via cannula  
11 to a solution of 472.9 mg (0.988 mmol) of ethyl 4-[(5,6-dihydro-5,5-  
12 dimethyl-8-(trifluoromethylsulfonyl)oxy-2-naphthalenyl)ethynyl]benzoate  
13 (Compound G) and 50 mg (0.04 mmol) of  
14 tetrakis(triphenylphosphine)palladium(0) in 4.0 ml of THF. The resulting  
15 solution was heated at 50°C for 45 minutes, cooled to room temperature and  
16 diluted with sat. aqueous NH<sub>4</sub>Cl. The mixture was extracted with EtOAc (40  
17 ml) and the combined organic layers were washed with water and brine.  
18 The organic phase was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo to a  
19 yellow oil. Purification by column chromatography (silica, 5% EtOAc-  
20 hexanes) yielded the title compound as a colorless solid. <sup>1</sup>H NMR (d6-  
21 acetone): δ 1.35 (6H, s), 1.40 (3H, t, J = 7.1 Hz), 2.36 (2H, d, J = 4.7 Hz),  
22 2.42 (3H, s), 4.38 (2H, q, J = 7.1 Hz), 5.99 (1H, t, J = 4.7 Hz), 7.25 (5H, m),  
23 7.35 (2H, m), 7.52 (2H, d, J = 8.5 Hz), 7.98 (2H, d, J = 8.5 Hz).

24 Ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-phenyl-2-naphthalenyl)ethynyl]benzoate  
25 (Compound 1a)

26 Employing the same general procedure as for the preparation of ethyl  
27 4-[(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-  
28 naphthalenyl)ethynyl]benzoate (Compound 1), 203.8 mg (0.43 mmol) of ethyl  
29 4-[(5,6-dihydro-5,5-dimethyl-8-(trifluoromethylsulfonyl)oxy-2-

1 naphthalenyl)ethynyl]benzoate (Compound G) was converted into the title  
2 compound (colorless solid) using 58.2 mg (0.36 ml, 0.69 mmol) of  
3 phenyllithium (1.8M solution in cyclohexane/Et<sub>2</sub>O), 116.1 mg (0.85 mmol) of  
4 zinc chloride and 13.8 mg (0.01 mmol) of  
5 tetrakis(triphenylphosphine)palladium(0). PMR (CDCl<sub>3</sub>):  $\delta$  1.36 (6H, s),  
6 1.40 (3H, t, J = 7.1 Hz), 2.37 (2H, d, J = 4.7 Hz), 4.38 (2H, q, J = 7.1 Hz),  
7 6.02 (1H, t, J = 4.7 Hz), 7.20 (1H, d, J = 1.5 Hz), 7.27 (1H, m), 7.39 (6H,  
8 m), 7.52 (2H, d, J = 8.2 Hz), 7.98 (2H, d, J = 8.2 Hz).

9 Ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(3-methylphenyl)-2-  
10 naphthalenyl)ethynyl]benzoate (Compound 2)

11 Employing the same general procedure as for the preparation of ethyl  
12 4-[(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-  
13 naphthalenyl)ethynyl]benzoate (Compound 1), 250.0 mg (0.522 mmol) of  
14 ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(trifluoromethylsulfonyl)oxy-2-  
15 naphthalenyl)ethynyl]benzoate (Compound G) was converted into the title  
16 compound (colorless solid) using 284.8 mg (2.090 mmol) of zinc chloride, 24  
17 mg (0.02 mmol) of tetrakis(triphenylphosphine)palladium(0) in 2.0 ml of  
18 THF, and 3-methylphenyl lithium (prepared by adding 201.2 mg (1.86 ml,  
19 3.14 mmol) of tert-butyllithium (1.7M solution in pentane) to a cold solution  
20 (-78°C) of 274.0 mg (1.568 mmol) of 3-methylbromobenzene in 2.0 ml of  
21 THF). <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  7.99 (2H, d, J = 8.4 Hz), 7.51 (2H, d, J = 8.4  
22 Hz), 7.39-7.14 (7H, m), 5.99 (1H, t, J = 4.7 Hz), 4.37 (2H, q, J = 7.1 Hz),  
23 2.60 (3H, s), 2.35 (2H, d, J = 4.7 Hz), 1.39 (3H, t, J = 7.1 Hz), 1.34 (6H, s).

24 Ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(2-methylphenyl)-2-  
25 naphthalenyl)ethynyl]benzoate (Compound 3)

26 Employing the same general procedure as for the preparation of ethyl  
27 4-[(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-  
28 naphthalenyl)ethynyl]benzoate (Compound 1), 200.0 mg (0.418 mmol) of  
29 ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(trifluoromethylsulfonyl)oxy-2-

1 naphthalenyl)ethynyl]benzoate (Compound G) was converted into the title  
2 compound (colorless solid) using 199.4 mg (1.463 mmol) of zinc chloride, 24  
3 mg (0.02 mmol) of tetrakis(triphenylphosphine)palladium(0) in 4.0 ml of  
4 THF, and 2-methylphenyl lithium (prepared by adding 133.9 mg (1.23 ml,  
5 2.09 mmol) of tert-butyllithium (1.7M solution in pentane) to a cold solution  
6 (-78°C) of 178.7 mg (1.045 mmol) of 2-methylbromobenzene in 2.0 ml of  
7 THF). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.97 (2H, d, J = 8.4 Hz), 7.50 (2H, d, J = 8.4  
8 Hz), 7.49-7.19 (6H, m), 6.81 (1H, d, J = 1.6 Hz), 5.89 (1H, t, J = 4.5 Hz),  
9 4.36 (2H, q, J = 7.1 Hz), 2.43-2.14 (2H, dq, J = 3.7, 5.4 Hz), 2.15 (3H, s),  
10 1.39-1.34 (9H, m).

11 Ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(3,5-dimethylphenyl)-2-  
12 naphthalenyl)ethynyl]benzoate (Compound 4)

13 Employing the same general procedure as for the preparation of ethyl  
14 4-[(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-  
15 naphthalenyl)ethynyl]benzoate (Compound 1), 250.0 mg (0.522 mmol) of  
16 ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(trifluoromethylsulfonyl)oxy-2-  
17 naphthalenyl)ethynyl]benzoate (Compound G) was converted into the title  
18 compound (colorless solid) using 249.0 mg (1.827 mmol) of zinc chloride, 24  
19 mg (0.02 mmol) of tetrakis(triphenylphosphine)palladium(0) in 2.0 ml of  
20 THF, and 3,5-dimethylphenyl lithium (prepared by adding 167.7 mg (1.54 ml,  
21 2.62 mmol) of tert-butyllithium (1.7M solution in pentane) to a cold solution  
22 (-78°C) of 249.0 mg (1.305 mmol) of 3,5-dimethylbromobenzene in 2.0 ml of  
23 THF). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.98 (2H, d, J = 8.4 Hz), 7.52 (2H, d, J = 8.4  
24 Hz), 7.40-7.33 (2H, m), 7.20 (1H, d, J = 1.6 Hz), 7.00 (1H, s), 6.97 (2H, s),  
25 5.97 (1H, t, J = 4.8 Hz), 4.37 (2H, q, J = 7.1 Hz), 2.36 (6H, s), 2.34 (2H, d, J  
26 = 4.8 Hz), 1.39 (3H, t, J = 7.1 Hz), 1.37 (6H, s).

27 Ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(4-ethylphenyl)-2-  
28 naphthalenyl)ethynyl]benzoate (Compound 5)

29 Employing the same general procedure as for the preparation of ethyl



1 4-[(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-  
2 naphthalenyl)ethynyl]benzoate (Compound 1), 250.0 mg (0.522 mmol) of  
3 ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(trifluoromethylsulfonyl)oxy-2-  
4 naphthalenyl)ethynyl]benzoate (Compound G) was converted into the title  
5 compound (colorless solid) using 249.0 mg (1.827 mmol) of zinc chloride, 24  
6 mg (0.02 mmol) of tetrakis(triphenylphosphine)palladium(0) in 2.0 ml of  
7 THF, and 4-ethylphenyl lithium (prepared by adding 167.7 mg (1.54 ml, 2.62  
8 mmol) of tert-butyllithium (1.7M solution in pentane) to a cold solution  
9 (-78°C) of 244.0 mg (1.305 mmol) of 4-ethylbromobenzene in 2.0 ml of THF).  
10 <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.99 (2H, d, J = 8.4 Hz), 7.51 (2H, d, J = 8.4 Hz),  
11 7.42 - 7.24 (7H, m), 5.99 (1H, t, J = 4.7 Hz), 4.37 (2H, q, J = 7.1 Hz), 2.71  
12 (2H, q, J = 7.6 Hz), 2.35 (2H, d, J = 4.7 Hz), 1.39 (3H, t, J = 7.1 Hz), 1.34  
13 (6H, s).

14 Ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(4-(1,1-dimethylethyl)phenyl)-2-  
15 naphthalenyl)ethynyl]benzoate (Compound 6)

16 Employing the same general procedure as for the preparation of ethyl  
17 4-[(5,6-dihydro-5,5-dimethyl-8-(2-thiazolyl)-2-naphthalenyl)ethynyl]benzoate  
18 (Compound 1), 250.0 mg (0.52 mmol) of ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-  
19 (trifluoromethylsulfonyl)oxy-2-naphthalenyl)ethynyl]benzoate (Compound G)  
20 was converted into the title compound (colorless solid) using 142.4 mg (1.045  
21 mmol) of zinc chloride and 4-tert-butylphenyl lithium (prepared by adding  
22 100.6 mg (0.97 ml, 1.57 mmol) of tert-butyllithium (1.5M solution in pentane)  
23 to a cold solution (-78°C) of 167.0 mg (0.78 mmol) of 4-tert-  
24 butylbromobenzene in 1.0 ml of THF). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.99 (2H, d, J  
25 = 8.4 Hz), 7.55 (2H, d, J = 8.4 Hz), 7.28-7.45 (7H, m), 6.02 (1H, t, J = 4.9  
26 Hz), 4.38 (2H, q, J = 7.2 Hz), 2.36 (2H, d, J = 4.9 Hz), 1.59 (3H, s), 1.40  
27 (3H, t, J = 7.2 Hz), 1.39 (9H, s), 1.35 (6H, s).

28 Ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(4-chlorophenyl)-2-  
29 naphthalenyl)ethynyl]benzoate (Compound 7)

1       Employing the same general procedure as for the preparation of ethyl  
2 4-[(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-  
3 naphthalenyl)ethynyl]benzoate (Compound 1), 250.0 mg (0.522 mmol) of  
4 ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(trifluoromethylsulfonyl)oxy-2-  
5 naphthalenyl)ethynyl]benzoate (Compound G) was converted into the title  
6 compound (colorless solid) using 249.0 mg (1.827 mmol) of zinc chloride, 24  
7 mg (0.02 mmol) of tetrakis(triphenylphosphine)palladium(0) in 2.0 ml of  
8 THF, and 4-chlorophenyl lithium (prepared by adding 167.7 mg (1.54 ml, 2.62  
9 mmol) of tert-butyllithium (1.7M solution in pentane) to a cold solution (-  
10 78°C) of 252.4 mg (1.305 mmol) of 4-chloro-1-bromobenzene in 2.0 ml of  
11 THF). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.98 (2H, d, J = 8.4 Hz), 7.53 (2H, d, J = 8.4  
12 Hz), 7.40-7.27 (6H, m), 7.12 (1H, d, J = 1.6 Hz), 6.00 (1H, t, J = 4.8 Hz),  
13 4.37 (2H, q, J = 7.1 Hz), 2.35 (2H, d, J = 4.8 Hz), 1.40 (2H, t, J = 7.1 Hz),  
14 1.34 (6H, s).

15 Ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(4-methoxyphenyl)-2-  
16 naphthalenyl)ethynyl]benzoate (Compound 8)

17       Employing the same general procedure as for the preparation of ethyl  
18 4-[(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-  
19 naphthalenyl)ethynyl]benzoate (Compound 1), 250.0 mg (0.522 mmol) of  
20 ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(trifluoromethylsulfonyl)oxy-2-  
21 naphthalenyl)ethynyl]benzoate (Compound G) was converted into the title  
22 compound (colorless solid) using 249.0 mg (1.827 mmol) of zinc chloride, 24  
23 mg (0.02 mmol) of tetrakis(triphenylphosphine)palladium(0) in 2.0 ml of  
24 THF, and 4-methoxyphenyl lithium (prepared by adding 167.7 mg (1.54 ml,  
25 2.62 mmol) of tert-butyllithium (1.7M solution in pentane) to a cold solution  
26 (-78°C) of 244.1 mg (1.305 mmol) of 4-methoxy-1-bromobenzene in 2.0 ml of  
27 THF). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.98 (2H, d, J = 8.5 Hz), 7.52 (2H, d, J = 8.6  
28 Hz), 7.40-7.21 (5H, m), 6.95 (2H, d, J = 8.7 Hz), 5.97 (1H, t, J = 4.7 Hz),  
29 4.37 (2H, q, J = 7.1 Hz), 4.34 (3H, s), 2.34 (2H, d, J = 4.7 Hz), 1.39 (3H, t, J

1 = 7.1 Hz), 1.34 (6H, s).

2 Ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(4-trifluoromethylphenyl)-2-

3 naphthalenyl)ethynyl]benzoate (Compound 9)

4 Employing the same general procedure as for the preparation of ethyl

5 4-[(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-

6 naphthalenyl)ethynyl]benzoate (Compound 1), 250.0 mg (0.522 mmol) of

7 ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(trifluoromethylsulfonyl)oxy-2-

8 naphthalenyl)ethynyl]benzoate (Compound G) was converted into the title

9 compound (colorless solid) using 249.0 mg (1.827 mmol) of zinc chloride, 24

10 mg (0.02 mmol) of tetrakis(triphenylphosphine)palladium(0) in 2.0 ml of

11 THF, and 4-trifluoromethylphenyl lithium (prepared by adding 167.7 mg

12 (1.54 ml, 2.62 mmol) of tert-butyllithium (1.7M solution in pentane) to a cold

13 solution (-78°C) of 296.6 mg (1.305 mmol) of 4-

14 trifluoromethylbromobenzene in 2.0 ml of THF). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.98

15 (2H, d, J = 8.5 Hz), 7.67 (2H, d, J = 8.3 Hz), 7.54 - 7.36 (6H, m), 7.10 (1H,

16 d, J = 1.6 Hz), 6.06 (1H, t, J = 4.8 Hz), 4.37 (2H, q, J = 7.1 Hz), 2.38 (2H,

17 d, J = 4.8 Hz), 1.39 (3H, t, J = 7.1 Hz), 1.35 (6H, s).

18 Ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(2-pyridyl)-2-

19 naphthalenyl)ethynyl]benzoate (Compound 10)

20 Employing the same general procedure as for the preparation of ethyl

21 4-[(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-

22 naphthalenyl)ethynyl]benzoate (Compound 1), 250.0 mg (0.52 mmol) of ethyl

23 4-[(5,6-dihydro-5,5-dimethyl-8-(trifluoromethylsulfonyl)oxy-2-

24 naphthalenyl)ethynyl]benzoate (Compound G) was converted into the title

25 compound (colorless solid) using 142.4 mg (1.045 mmol) of zinc chloride and

26 2-lithiopyridine (prepared by the addition of 100.6 mg (0.97 ml, 1.57 mmol)

27 of tert-butyllithium (1.5M solution in pentane) to a cold solution (-78°C) of

28 123.8 mg (0.784 mmol) of 2-bromopyridine in 1.0 ml of THF). <sup>1</sup>H NMR

29 (d<sub>6</sub>-acetone): δ 8.64 (1H, m), 7.99 (2H, d, J = 8.5 Hz), 7.85 (1H, ddd, J =

1 1.8, 7.7, 9.5 Hz), 7.58 (2H, d, J = 8.4 Hz), 7.50 (1H, d, J = 7.7 Hz), 7.47 (2  
2 H, d, J = 1.1 Hz), 7.35 (2H, m), 6.32 (1H, t, J = 4.8 Hz), 4.34 (2H, q, J =  
3 7.2 Hz), 2.42 (2H, d, J = 7.4 Hz), 1.35 (3H, t, J = 7.0 Hz), 1.35 (6H, s).

4 Ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(3-pyridyl)-2-  
5 naphthalenyl)ethynyl]benzoate (Compound 11)

6 Employing the same general procedure as for the preparation of ethyl  
7 4-[(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-  
8 naphthalenyl)ethynyl]benzoate (Compound 1), 170.0 mg (0.35 mmol) of ethyl  
9 4-[(5,6-dihydro-5,5-dimethyl-8-(trifluoromethylsulfonyl)oxy-2-  
10 naphthalenyl)ethynyl]benzoate (Compound G) was converted into the title  
11 compound (colorless solid) using 142.4 mg (1.045 mmol) of zinc chloride and  
12 3-lithiopyridine (prepared by the addition of 100.2 mg (0.92 ml, 1.56 mmol)  
13 of tert-butyllithium (1.5M solution in pentane) to a cold solution (-78°C) of  
14 123.8 mg (0.784 mmol) of 3-bromopyridine in 1.0 ml of THF). <sup>1</sup>H NMR  
15 (CDCl<sub>3</sub>): δ 8.63-8.61 (2H, dd, J = 1.7 Hz), 7.99 2H, d, J = 8.4 Hz), 7.67  
16 (1H, dt, J = 7.9 Hz), 7.52 (2H, d, J = 8.4 Hz), 7.43-7.34 (3H, m), 7.10 (1H,  
17 d, J = 1.6 Hz), 6.07 (1H, t, J = 4.7 Hz), 4.37 (2H, q, J = 7.1 Hz), 2.40 (2H,  
18 d, J = 4.7 Hz), 1.390 (3H, t, J = 7.1 Hz), 1.36 (6H, s).

19 Ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(2-methyl-5-pyridyl)-2-  
20 naphthalenyl)ethynyl]benzoate (Compound 12)

21 Employing the same general procedure as for the preparation of ethyl  
22 4-[(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-  
23 naphthalenyl)ethynyl]benzoate (Compound 1), 250.0 mg (0.522 mmol) of  
24 ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(trifluoromethylsulfonyl)oxy-2-  
25 naphthalenyl)ethynyl]benzoate (Compound G) was converted into the title  
26 compound (colorless solid) using 142.4 mg (1.045 mmol) of zinc chloride and  
27 2-methyl-5-lithiopyridine (prepared by the addition of 100.5 mg (0.92 ml,  
28 1.57 mmol) of tert-butyllithium (1.7 M solution in pentane) to a cold solution  
29 (-78°C) of 134.8 mg (0.784 mmol) of 2-methyl-5-bromopyridine in 1.0 ml of

1 THF).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  8.50 (1H, d,  $J = 2.2$  Hz), 7.99 (2H, d,  $J = 8.3$   
2 Hz), 7.56 (1H, dd,  $J = 2.3, 8.0$  Hz), 7.53 (2H, d,  $J = 8.4$  Hz), 7.43 (1H, dd,  $J$   
3 = 2.3, 8.0 Hz), 7.37 (2H, d,  $J = 8.0$  Hz), 7.21 (1H, d,  $J = 8.1$  Hz), 7.11 (1H,  
4 d,  $J = 1.5$  Hz), 6.04 (1H, t,  $J = 4.7$  Hz), 4.38 (2H, q,  $J = 7.2$  Hz), 2.63 (3H,  
5 s), 2.38 (2H, d,  $J = 4.6$  Hz), 1.40 (3H, t,  $J = 7.1$  Hz), 1.35 (6H, s).

6 Ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(3-((2,2-dimethylethyl)-  
7 dimethylsiloxy)phenyl)-2-naphthalenyl)ethynyl]benzoate (Compound H)

8       Employing the same general procedure as for the preparation of ethyl  
9 4-[(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-  
10 naphthalenyl)ethynyl]benzoate (Compound G), 150.0 mg (0.314 mmol) of  
11 ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(trifluoromethylsulfonyl)oxy-2-  
12 naphthalenyl)ethynyl]benzoate (Compound G) was converted into the title  
13 compound (colorless solid) using 150.0 mg (1.10 mmol) of zinc chloride, 24  
14 mg (0.02 mmol) of tetrakis(triphenylphosphine)palladium(0) in 2.0 ml of  
15 THF, and 3-((2,2-dimethylethyl)dimethylsiloxy)phenyl lithium (prepared by  
16 adding 100.2 mg (0.92 ml, 1.564 mmol) of tert-butyllithium (1.7M solution in  
17 pentane) to a cold solution ( $-78^\circ\text{C}$ ) of 226.0 mg (0.787 mmol) of 3-((2,2-  
18 dimethylethyl)dimethylsiloxy)bromobenzene in 2.0 ml of THF).  $^1\text{H}$  NMR  
19 ( $\text{CDCl}_3$ ):  $\delta$  7.98 (2H, d,  $J = 8.4$  Hz), 7.51 (2H, d,  $J = 8.4$  Hz), 7.40-7.22 (4H,  
20 m), 6.95 (1H, d,  $J = 7.6$  Hz), 6.84-6.82 (2H, m), 6.00 (1H, t,  $J = 4.7$  Hz),  
21 4.37 (2H, q,  $J = 7.1$  Hz), 2.35 (2H, d,  $J = 4.7$  Hz), 1.39 (3H, t,  $J = 7.1$  Hz),  
22 1.34 (3H, s), 0.99 (9H, s), 0.23 (6H, s).

23 Ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(4-((2,2-dimethylethyl)-  
24 dimethylsiloxy)phenyl)-2-naphthalenyl)ethynyl]benzoate (Compound I)

25       Employing the same general procedure as for the preparation of ethyl  
26 4-[(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-  
27 naphthalenyl)ethynyl]benzoate (Compound 1), 210.0 mg (0.439 mmol) of  
28 ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(trifluoromethylsulfonyl)oxy-2-  
29 naphthalenyl)ethynyl]benzoate (Compound G) was converted into the title

1 compound (colorless solid) using 209.0 mg (1.53 mmol) of zinc chloride, 24  
2 mg (0.02 mmol) of tetrakis(triphenylphosphine)palladium(0) in 2.0 ml of  
3 THF, and 4-((2,2-dimethylethyl)dimethylsiloxy)phenyl lithium (prepared by  
4 adding 140.3 mg (1.30 ml, 2.19 mmol) of tert-butyllithium (1.7M solution in  
5 pentane) to a cold solution (-78°C) of 315.0 mg (1.09 mmol) of 4-((2,2-  
6 dimethylethyl)dimethylsiloxy)bromobenzene in 2.0 ml of THF). <sup>1</sup>H NMR  
7 (CDCl<sub>3</sub>): δ 7.98 (2H, d, J = 8.4 Hz), 7.51 (2H, d, J = 8.4 Hz), 7.39-7.25 (3H,  
8 m), 7.21 (2H, d, J = 8.5 Hz), 5.87 (2H, d, J = 8.5 Hz), 5.96 (1H, t, J = 4.7  
9 Hz), 4.37 (2H, q, J = 7.1 Hz), 2.33 (2H, d, J = 4.7 Hz), 1.39 (3H, t, J = 7.1  
10 Hz), 1.33 (6H, s), 1.01 (9H, s), 0.25 (6H, s).

11 Ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(3-hydroxyphenyl)-2-  
12 naphthalenyl)ethynyl]benzoate (Compound 13)

13 To a solution of ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(3-((2,2-  
14 dimethylethyl)-dimethylsiloxy)-phenyl)-2-naphthalenyl)ethynyl]benzoate  
15 (Compound H) 60.0 mg (0.114 mmol) in 1.0 ml of THF at room temperature  
16 was added 91.5 mg (0.35 ml, 0.35 mmol) of tetrabutylammonium fluoride (1 M  
17 solution in THF). After stirring overnight, the solution was diluted with  
18 EtOAc and washed with H<sub>2</sub>O and saturated aqueous NaCl, before being  
19 dried over MgSO<sub>4</sub>. Removal of the solvents under reduced pressure,  
20 followed by column chromatography (4:1, Hexanes:EtOAc) afforded the title  
21 compound as a colorless solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.98 (2H, d, J = 7.8  
22 Hz), 7.52 (2H, d, J = 8.3 Hz), 7.39 - 7.21 (4H, m), 6.93 (1H, d, J = 7.5 Hz),  
23 6.84 (1H, d, 7.1 Hz), 6.83 (1H, s), 6.01 1H, t, J = 4.7 Hz), 4.91 (1H, s), 4.39  
24 (2H, q, J = 7.1 Hz), 2.35 (2H, d, J = 4.7 Hz), 1.39 (3H, t, J = 7.1 Hz), 1.34  
25 (6H, s).

26 Ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(4-hydroxyphenyl)-2-  
27 naphthalenyl)ethynyl]benzoate (Compound 14)

28 To a solution of ethyl 4-[(5,6-dihydro-5,5- dimethyl-8-(4-((2,2-  
29 dimethylethyl)- dimethylsiloxy)phenyl)-2-naphthalenyl)ethynyl]benzoate

(Compound I) 50.0 mg (0.095 mmol) in 1.0 ml of THF at room temperature was added 73.2 mg (0.29 ml, 0.29 mmol) of tetrabutylammonium fluoride (1 M solution in THF). After stirring overnight, the solution was diluted with EtOAc and washed with H<sub>2</sub>O and saturated aqueous NaCl, before being dried over MgSO<sub>4</sub>. Removal of the solvents under reduced pressure, followed by column chromatography (4:1, Hexanes:EtOAc) afforded the title compound as a colorless solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.98 (2H, d, J = 8.2 Hz), 7.52 (2H, d, J = 8.3 Hz), 7.41 - 7.20 (5H, m), 6.88 (2H, d, J = 8.4 Hz), 5.96 (1H, t, J = 4.5 Hz), 4.37 (2H, q, J = 7.1 Hz), 2.34 (2H, d, J = 4.5 Hz), 1.39 (3H, t, J = 7.1 Hz), 1.34 (6H, s).

Ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(5-methylthiazol-2-yl)-2-naphthalenyl)ethynyl] benzoate (Compound 15)

Employing the same general procedure as for the preparation of ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-naphthalenyl)ethynyl]benzoate (Compound 1), 264.0 mg (0.552 mmol) of ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(trifluoromethylsulfonyl)oxy-2-naphthalenyl)ethynyl]benzoate (Compound G) was converted into the title compound (colorless solid) using 150.0 mg (1.10 mmol) of zinc chloride, 14 mg (0.012 mmol) of tetrakis(triphenylphosphine)palladium(0) in 4.0 ml of THF, and 5-methylthiazol-2-yl lithium (prepared by adding 53.2 mg (0.53 ml, 0.83 mmol) of n-butyllithium (1.55 M solution in hexanes) to a cold solution (-78°C) of 82.0 mg (0.83 mmol) of 5-methylthiazole in 5.0 ml of THF). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.99 (2H, d, J = 7.8 Hz), 7.88 (1H, d, J = 1.5 Hz), 7.55 (2H, d, J = 7.8 Hz), 7.54 (1H, s), 7.45 (1H, dd, J = 1.5, 8.0 Hz), 7.35 (1H, d, J = 7.9 Hz), 6.48 (1H, t, J = 4.8 Hz), 4.38 (2H, q, J = 7.1 Hz), 2.51 (3H, s), 2.38 (2H, d, J = 4.8 Hz), 1.40 (3H, s), 1.32 (6H, s).

Ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(2-thiazolyl)-2-naphthalenyl)ethynyl]benzoate (Compound 15a)

A solution of 2-lithiothiazole was prepared by the addition of 41.2 mg

1 (0.42 ml, 0.63 mmol) of n-butyl- lithium (1.5M solution in hexanes) to a cold  
2 solution (-78 °C) of 53.4 mg (0.63 mmol) of thiazole in 1.0 ml of THF. The  
3 solution was stirred at for 30 minutes and then a solution of 113.9 mg (0.84  
4 mmol) of zinc chloride in 1.5 ml of THF was added. The resulting solution  
5 was warmed to room temperature, stirred for 30 minutes and then the  
6 organozinc was added via cannula to a solution of 200.0 mg (0.42 mmol) of  
7 ethyl 4-[(5,6- dihydro-5,5-dimethyl-8-(trifluoromethylsulfonyl)oxy-2-  
8 naphthalenyl)ethynyl]benzoate (Compound G) and 12.4 mg (0.01 mmol) of  
9 tetrakis(triphenylphosphine)palladium(0) in 1.5 ml of THF. The resulting  
10 solution was heated at 50°C for 45 minutes, cooled to room temperature and  
11 diluted with sat. aqueous NH<sub>4</sub>Cl. The mixture was extracted with EtOAc (40  
12 ml) and the combined organic layers were washed with water and brine. The  
13 organic phase was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo to a yellow  
14 oil. Purification by column chromatography (silica, 20% EtOAc-hexanes)  
15 yielded the title compound as a colorless oil. PMR (CDCl<sub>3</sub>): δ 1.35 (6H, s),  
16 1.40 (3H, t, J = 7.1 Hz), 2.42 (2H, d, J = 4.8 Hz), 4.38 (2H, q, J = 7.1 Hz),  
17 6.57 (1H, t, J = 4.8 Hz), 7.33 (1H, d, J = 3.3 Hz), 7.36 (1H, d, J = 8.0 Hz),  
18 7.46 (1H, dd, J = 1.7 , 8.1 Hz), 7.55 (2H, d, J = 8.4 Hz), 7.87 (1H, d, J = 1.7  
19 Hz), 7.92 (1H, d, J = 3.3 Hz), 8.00 (2H, d, J = 8.4 Hz).  
20 Ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(4-methylthiazol-2-yl)-2-  
21 naphthalenyl)ethynyl] benzoate (Compound 16)

22       Employing the same general procedure as for the preparation of ethyl  
23 4-[(5,6-dihydro-5,5-dimethyl-8-(4- methylphenyl)-2-  
24 naphthalenyl)ethynyl]benzoate (Compound 1), 295.0 mg (0.617 mmol) of  
25 ethyl 4-[(5,6-dihydro-5,5- dimethyl-8-(trifluoromethylsulfonyl)oxy-2-  
26 naphthalenyl)ethynyl]benzoate (Compound G) was converted into the title  
27 compound (colorless-solid)-using 168.0 mg (1.23 mmol) of zinc chloride, 16  
28 mg (0.014 mmol) of tetrakis(triphenylphosphine)palladium(0) in 6.0 ml of  
29 THF, and 4-methylthiazol-2-yl lithium (prepared by adding 59.6 mg (0.60 ml,



1 0.93 mmol) of n- butyllithium (1.55 M solution in hexanes) to a cold solution  
2 (-78°C) of 92.0 mg (0.93 mmol) of 4-methylthiazole in 6.0 ml of THF). <sup>1</sup>H  
3 NMR (CDCl<sub>3</sub>): δ 8.00 (2H, d, J = 8.4 Hz), 7.80 (1H, d, J = 1.7 Hz), 7.55  
4 (2H, d, J = 8.4 Hz), 7.45 (1H, dd, J = 1.7, 8.0 Hz), 7.35 (1H, d, J = 8.0 Hz),  
5 6.87 (1H, s), 6.52 (1H, t, J = 4.7 Hz), 4.37 (2H, q, J = 7.2 Hz), 2.54 (3H, s),  
6 2.39 (2H, d, J = 4.7 Hz), 1.40 (3H, t, J = 7.2 Hz), 1.33 (3H, s).

7 Ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(4,5-dimethylthiazol-2-yl)-2-naphthalenyl)  
8 ethynyl] benzoate (Compound 17)

9       Employing the same general procedure as for the preparation of ethyl  
10 4-[(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-  
11 naphthalenyl)ethynyl]benzoate (Compound 1), 200.0 mg (0.418 mmol) of  
12 ethyl 4-[(5,6-dihydro-5,5- dimethyl-8-(trifluoromethylsulfonyl)oxy-2-  
13 naphthalenyl)ethynyl]benzoate (Compound G) was converted into the title  
14 compound (colorless solid) using 110.0 mg (0.84 mmol) of zinc chloride, 12  
15 mg (0.011 mmol) of tetrakis(triphenylphosphine)palladium(0) in 2.0 ml of  
16 THF, and 4,5-dimethylthiazol-2-yl lithium (prepared by adding 40.2 mg (0.39  
17 ml, 0.63 mmol) of n- butyllithium (1.55 M solution in hexanes) to a cold  
18 solution (-78°C) of 71.0 mg (0.63 mmol) of 4,5-dimethylthiazole in 2.0 ml of  
19 THF). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 8.00 (2H, d, J = 8.4 Hz), 7.82 (1H, d, J = 1.7  
20 Hz), 7.54 (2H, d, J = 8.4 Hz ), 7.43 (1H, dd, J = 1.7, 8.0 Hz), 7.33 91H, d, J  
21 = 8.0 Hz), 6.45 (1H, t, J = 4.9 Hz), 4.38 (2H, q, J = 7.1 Hz), 2.41 (3H, s),  
22 2.40 (3H, s), 2.37 (2H, d, J = 4.9 Hz), 1.40 (3H, t, J = 7.1 Hz), 1.32 (6H, s).

23 4-[(5,6-Dihydro-5,5-dimethyl-8-(2-methyl-5-pyridyl)-2-  
24 naphthalenyl)ethynyl]benzoic acid (Compound 18)

25       A solution of 81.7 mg (0.194 mmol) of ethyl 4- [(5,6-dihydro-5,5-  
26 dimethyl-8-(2-methyl-5-pyridyl)-2- naphthalenyl)ethynyl]benzoate (Compound  
27 12) and 40.7 mg (0.969 mmol) of LiOH·H<sub>2</sub>O in 3 ml of THF/water (3:1, v/v),  
28 was stirred overnight at room temperature. The reaction was quenched by  
29 the addition of saturated aqueous NH<sub>4</sub>Cl and extracted with EtOAc. The

1 combined organic layers were washed with water and brine, dried over  
2  $\text{Na}_2\text{SO}_4$  and concentrated in vacuo to give the title compound as a colorless  
3 solid.  $^1\text{H}$  NMR ( $\text{d}_6$ -DMSO):  $\delta$  8.41 (1H, d,  $J$  = 1.9 Hz), 7.90 (2H, d,  $J$  =  
4 8.3 Hz), 7.63 (1H, dd,  $J$  = 2.3, 7.9 Hz), 7.59 (2H, d,  $J$  = 8.3 Hz), 7.49 (2H,  
5 m), 7.33 (1H, d,  $J$  = 7.8 Hz), 6.95 (1H, s), 6.11 (1H, t,  $J$  = 4.5 Hz), 2.52 (3H,  
6 s), 2.37 (2H, d,  $J$  = 4.6 Hz), 1.31 (6H, s).

7 4-[(5,6-Dihydro-5,5-dimethyl-8-(2-pyridyl)-2-naphthalenyl)ethynyl]benzoic  
8 acid (Compound 19)

9 A solution of 80.0 mg (0.196 mmol) of ethyl 4-[(5,6-dihydro-5,5-  
10 dimethyl-8-(2-pyridyl)-2-naphthalenyl)ethynyl]benzoate (Compound 10) and  
11 20.6 mg (0.491 mmol) of  $\text{LiOH}\cdot\text{H}_2\text{O}$  in 3 ml of THF/water (3:1, v/v), was  
12 stirred overnight at room temperature. The reaction was quenched by the  
13 addition of saturated aqueous  $\text{NH}_4\text{Cl}$  and extracted with EtOAc. The  
14 combined organic layers were washed with water and brine, dried over  
15  $\text{Na}_2\text{SO}_4$  and concentrated in vacuo to give the title compound as a colorless  
16 solid.  $^1\text{H}$  NMR ( $\text{d}_6$ -DMSO):  $\delta$  8.64 (1H, m), 7.94 (2H, d,  $J$  = 8.3 Hz), 7.87  
17 (1H, dt,  $J$  = 1.7, 7.8 Hz), 7.58 (2H, d,  $J$  = 8.3 Hz), 7.50 (1H, d,  $J$  = 8.2 Hz),  
18 7.47 (2H, s), 7.37 (1H, m), 7.25 (1H, s), 6.30 (1H, t,  $J$  = 4.6 Hz), 2.39 (2H,  
19 d,  $J$  = 4.6 Hz), 1.31 (6H, s).

20 4-[(5,6-Dihydro-5,5-dimethyl-8-(3-methylphenyl)-2-  
21 naphthalenyl)ethynyl]benzoic acid (Compound 20)

22 To a solution of ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(3-  
23 methylphenyl)-2-naphthalenyl)ethynyl]benzoate (Compound 2) 30.0mg (0.071  
24 mmol) in 3 ml of EtOH and 2 ml of THF was added 28.0 mg (0.70 mmol, 0.7  
25 ml) of NaOH (1.0 M aqueous solution). The solution was heated to  $50^\circ\text{C}$  for  
26 2 hours, cooled to room temperature, and acidified with 10% HCl.  
27 Extraction with EtOAc, followed by drying over  $\text{Na}_2\text{SO}_4$ , and removal of the  
28 solvents under reduced pressure afforded the title compound as a colorless  
29 solid.  $^1\text{H}$  NMR (DMSO):  $\delta$  7.90 (2H, d,  $J$  = 8.5 Hz), 7.59 (2H, d,  $J$  = 8.5

1 Hz), 7.46 (2H, s), 7.32-7.13 (4H, m), 7.10 (1H, s), 6.98 (1H, t, J = 4.5 Hz),  
2 2.34 (3H, s), 2.31 (2H, d, J = 4.5 Hz), 1.30 (6H, s).  
3 4-[(5,6-Dihydro-5,5-dimethyl-8-(4-ethylphenyl)-2-naphthalenyl)ethynyl]benzoic  
4 acid (Compound 21)

5 To a solution of ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(4-ethylphenyl)-2-  
6 naphthalenyl)ethynyl]benzoate (Compound 5) 47.0mg (0.108 mmol) in 3 ml  
7 of EtOH and 2 ml of THF was added 28.0 mg (0.70 mmol, 0.7 ml) of NaOH  
8 (1.0 M aqueous solution). The solution was heated to 50°C for 2 hours,  
9 cooled to room temperature, and acidified with 10% HCl. Extraction with  
10 EtOAc, followed by drying over Na<sub>2</sub>SO<sub>4</sub>, and removal of the solvents under  
11 reduced pressure afforded the title compound as a colorless solid. 1H NMR  
12 (DMSO): δ 7.90 (2H, d, J = 8.3 Hz), 7.59 (2H, d, J = 8.3 Hz), 7.46 (2H, s),  
13 7.29-7.21 (4H, m), 7.02 (1H, s), 6.01 (1H, t, J = 4.5 Hz), 2.64 (2H, q, J = 7.5  
14 Hz), 2.33 (2H, d, J = 4.5 Hz), 1.29 (6H, s), 1.22 (3H, t, J = 7.5 Hz).

15 4-[(5,6-Dihydro-5,5-dimethyl-8-(4-methoxyphenyl)-2-  
16 naphthalenyl)ethynyl]benzoic acid (Compound 22)

17 To a solution of ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(4-  
18 methoxyphenyl)-2-naphthalenyl)ethynyl]benzoate (Compound 8) 80.0 mg  
19 (0.183 mmol) in 3 ml of EtOH and 2 ml of THF was added 40.0 mg (1.00  
20 mmol, 1.0 ml) of NaOH (1.0 M aqueous solution). The solution was heated  
21 to 50°C for 2 hours, cooled to room temperature, and acidified with 10%  
22 HCl. Extraction with EtOAc, followed by drying over Na<sub>2</sub>SO<sub>4</sub>, and removal  
23 of the solvents under reduced pressure afforded the title compound as a  
24 colorless solid. 1H NMR (DMSO): δ 7.90 (2H, d, J = 8.3 Hz), 7.60 (2H, d,  
25 J = 8.3 Hz), 7.45 (2H, s), 7.24 (2H, d, J = 8.6 Hz), 7.02-6.89 (3H, m), 5.98  
26 (1H, t, J = 4.4 Hz), 3.79 (3H, s), 2.31 (2H, d, J = 4.7 Hz), 1.29 (6H, s).

27 4-[(5,6-Dihydro-5,5-dimethyl-8-(4-trifluoromethylphenyl)-2-  
28 naphthalenyl)ethynyl]benzoic acid (Compound 23)

29 To a solution of ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(4-

1 trifluoromethylphenyl)-2-naphthalenyl)ethynyl]benzoate (Compound 9) 70.0  
2 mg (0.148 mmol) in 3 ml of EtOH and 2 ml of THF was added 60.0 mg (1.50  
3 mmol, 1.50 ml) of NaOH (1.0 M aqueous solution). The solution was heated  
4 to 50°C for 2 hours, cooled to room temperature, and acidified with 10%  
5 HCl. Extraction with EtOAc, followed by drying over Na<sub>2</sub>SO<sub>4</sub>, and removal  
6 of the solvents under reduced pressure afforded the title compound as a  
7 colorless solid. <sup>1</sup>H NMR (DMSO): δ 7.90 (2H, d, J = 8.3 Hz), 7.80 (2H, d,  
8 J = 8.1 Hz), 7.61-7.47 (6H, m), 6.97 (2H, s), 6.16 (1H, t, J = 4.5 Hz), 2.37  
9 (2H, d, J = 4.6 Hz), 1.30 (6H, s).

10 4-[(5,6-Dihydro-5,5-dimethyl-8-(3,5-dimethylphenyl)-2-  
11 naphthalenyl)ethynyl]benzoic acid (Compound 24)

12 To a solution of ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(3,5-  
13 dimethylphenyl)-2-naphthalenyl)ethynyl]-benzoate (Compound 4) 90.0 mg  
14 (0.207 mmol) in 3 ml of EtOH and 2 ml of THF was added 48.0 mg (1.20  
15 mmol, 1.20 ml) of NaOH (1.0 M aqueous solution). The solution was heated  
16 to 50°C for 2 hours, cooled to room temperature, and acidified with 10%  
17 HCl. Extraction with EtOAc, followed by drying over Na<sub>2</sub>SO<sub>4</sub>, and removal  
18 of the solvents under reduced pressure afforded the title compound as a  
19 colorless solid. <sup>1</sup>H NMR (DMSO): δ 7.90 (2H, d, J = 8.2 Hz), 7.59 (2H, d,  
20 J = 8.2 Hz), 7.45 (2H, s), 7.00 (1H, s), 6.97 (1H, s), 5.97 (1H, t, J = 4.5 Hz),  
21 2.31 (2H, d, J = 4.5 Hz), 2.30 (6H, s), 1.29 (6H, s).

22 4-[(5,6-Dihydro-5,5-dimethyl-8-(4-chlorophenyl)-2-  
23 naphthalenyl)ethynyl]benzoic acid (Compound 25)

24 To a solution of ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(4-  
25 chlorophenyl)-2-naphthalenyl)ethynyl]benzoate (Compound 7) 80.0 mg (0.181  
26 mmol) in 3 ml of EtOH and 2 ml of THF was added 48.0 mg (1.20 mmol,  
27 1.20 ml) of NaOH (1.0 M aqueous solution). The solution was heated to  
28 50°C for 2 hours, cooled to room temperature, and acidified with 10% HCl.  
29 Extraction with EtOAc, followed by drying over Na<sub>2</sub>SO<sub>4</sub>, and removal of the

1 solvents under reduced pressure afforded the title compound as a colorless  
2 solid. <sup>1</sup>H NMR (DMSO): δ 7.90 (2H, d, J = 8.3 Hz), 7.60 (2H, d, J = 8.3  
3 Hz), 7.51-7.48 (4H, m), 7.34 (2H, d, J = 8.4 Hz), 6.97 (1H, s), 6.07 (1H, t, J  
4 = 4.5 Hz), 2.34 (2H, d, J = 4.6 Hz), 1.29 (6H, s).

5 4-[(5,6-Dihydro-5,5-dimethyl-8-(3-pyridyl)-2-naphthalenyl)ethynyl]benzoic acid  
6 (Compound 26)

7 To a solution of ethyl 4-[(5,6-dihydro-5,5- dimethyl-8-(3-pyridyl)-2-  
8 naphthalenyl)ethynyl]benzoate (Compound 11) 45.0 mg (0.110 mmol) in 3 ml  
9 of EtOH and 2 ml of THF was added 48.0 mg (1.20 mmol, 1.20 ml) of  
10 NaOH (1.0 M aqueous solution). The solution was heated to 50°C for 2  
11 hours, cooled to room temperature, and acidified with 10% HCl. Extraction  
12 with EtOAc, followed by drying over Na<sub>2</sub>SO<sub>4</sub>, and removal of the solvents  
13 under reduced pressure afforded the title compound as a colorless solid. <sup>1</sup>H  
14 NMR (DMSO): δ 8.60 (1H, d, J = 4.6 Hz), 8.55 (1H, s), 7.90 (2H, d, J =  
15 8.3 Hz), 7.76 (1H, d, J = 7.5 Hz), 7.60 (2H, d, J = 8.3 Hz), 7.51-7.46 (3H,  
16 m), 6.94 (1H, s), 6.14 (1H, t, J = 4.5 Hz), 2.37 (2H, d, J = 4.5 Hz), 1.31 (6H,  
17 s).

18 4-[(5,6-Dihydro-5,5-dimethyl-8-(2-methylphenyl)-2-  
19 naphthalenyl)ethynyl]benzoic acid (Compound 27)

20 To a solution of ethyl 4-[(5,6-dihydro-5,5- dimethyl-8-(2-  
21 methylphenyl)-2-naphthalenyl)ethynyl]- benzoate (Compound 3) 80.0 mg  
22 (0.190 mmol) in 3 ml of EtOH and 2 ml of THF was added 60.0 mg (1.50  
23 mmol, 1.50 ml) of NaOH (1.0 M aqueous solution). The solution was heated  
24 to 50°C for 2 hours, cooled to room temperature, and acidified with 10%  
25 HCl. Extraction with EtOAc, followed by drying over Na<sub>2</sub>SO<sub>4</sub>, and removal  
26 of the solvents under reduced pressure afforded the title compound as a  
27 colorless solid. <sup>1</sup>H NMR (DMSO): δ 7.89(2H, d, J = 8.4 Hz), 7.57 (2H, d, J  
28 = 8.4 Hz), 7.46 (2H, s), 7.29-7.14 (4H, m), 6.59 (1H, s), 5.90 (1H, t, J = 4.7  
29 Hz), 2.39 (2H, m), 2.60 (3H, s), 1.39 (3H, s), 1.29 (3H, s).

1 4-[(5,6-Dihydro-5,5-dimethyl-8-(3-hydroxyphenyl)-2-  
2 naphthalenyl)ethynyl]benzoic acid (Compound 28)

3 To a solution of ethyl 4-[(5,6-dihydro-5,5- dimethyl-8-(3-((2,2-  
4 dimethylethyl)- dimethylsiloxy)phenyl)-2-naphthalenyl)ethynyl]benzoate  
5 (Compound H) 40.0 mg (0.076 mmol) in 3 ml of EtOH and 2 ml of THF was  
6 added 40.0 mg (1.00 mmol, 1.00 ml) of NaOH (1.0 M aqueous solution).  
7 The solution was heated to 50°C for 2 hours, cooled to room temperature,  
8 and acidified with 10% HCl. Extraction with EtOAc, followed by drying over  
9 Na<sub>2</sub>SO<sub>4</sub>, and removal of the solvents under reduced pressure afforded the  
10 title compound as a colorless solid. <sup>1</sup>H NMR (d<sub>6</sub>-acetone): δ 7.90 (2H, d, J  
11 = 8.3 Hz), 7.49 (2H, d, J = 8.4 Hz), 7.35 (2H, s), 7.15-7.07 (2H, m), 6.77-6.69  
12 (3H, m), 5.92 (1H, t, J = 4.7 Hz), 2.25 (2H, d, J = 4.7 Hz), 1.23 (6H, s).

13 4-[(5,6-Dihydro-5,5-dimethyl-8-(4-hydroxyphenyl)-2-  
14 naphthalenyl)ethynyl]benzoic acid (Compound 29)

15 To a solution of ethyl 4-[(5,6-dihydro-5,5- dimethyl-8-(4-((2,2-  
16 dimethylethyl)- dimethylsiloxy)phenyl)-2-naphthalenyl)ethynyl]benzoate  
17 (Compound I) 75.0 mg (0.143 mmol) in 3 ml of EtOH and 2 ml of THF was  
18 added 60.0 mg (1.50 mmol, 1.50 ml) of NaOH (1.0 M aqueous solution).  
19 The solution was heated to 50°C for 2 hours, cooled to room temperature,  
20 and acidified with 10% HCl. Extraction with EtOAc, followed by drying over  
21 Na<sub>2</sub>SO<sub>4</sub>, and removal of the solvents under reduced pressure afforded the  
22 title compound as a colorless solid. <sup>1</sup>H NMR (d<sub>6</sub>-acetone): δ 8.01 (2H, d, J  
23 = 8.3 Hz), 7.59 (2H, d, J = 8.4 Hz), 7.45 (2H, s), 7.20-7.17 (3H, m), 6.92-6.89  
24 (2H, m), 5.97 (1H, t, J = 4.7 Hz), 2.35 (2H, d, J = 4.7 Hz), 1.34 (6H, s).

25 4-[(5,6-Dihydro-5,5-dimethyl-8-(5-methylthiazol-2-yl)-2-naphthalenyl)ethynyl]  
26 benzoic acid (Compound 30)

27 To a solution of ethyl 4-[5,6-dihydro-5,5-dimethyl-8-(5-methylthiazol-2-  
28 yl)-2-naphthalenyl]ethynylbenzoate (Compound 15) (100 mg, 0.23 mmol) and  
29 4 ml of EtOH at room temperature was added aqueous NaOH (1 ml, 1 M, 1

mmol). The resulting solution was warmed to 50°C for 1 hour and concentrated in vacuo. The residue was suspended in a solution of CH<sub>2</sub>Cl<sub>2</sub> and ether (5:1) and acidified to pH 5 with 1M aqueous HCl. The layers were separated and the organic layer was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and the solvents removed under reduced pressure to give the title compound as a white solid. <sup>1</sup>H NMR (d<sub>6</sub>-DMSO): δ 7.96 (1H, d, J = 1.7 Hz), 7.95 (2H, d, J = 8.0 Hz), 7.65 (2H, d, J = 8.0 Hz), 7.64 (1H, s), 7.53 (1H, dd, J = 1.7, 8.0 Hz), 7.46 (1H, d, J = 8.0 Hz), 6.59 (1H, t, J = 4.5 Hz), 2.50 (3H, s), 2.39 (2H, d, J = 4.5 Hz), 1.27 (6H, s).

4-[(5,6-dihydro-5,5-dimethyl-8-(2-thiazolyl)-2-naphthalenyl)ethynyl]benzoic acid (Compound 30a)

A solution of 33.9 mg (0.08 mmol) of ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(2-thiazolyl)-2-naphthalenyl)ethynyl]benzoate (Compound 15a) and 8.5 mg (0.20 mmol) of LiOH·H<sub>2</sub>O in 3 ml of THF/water (3:1, v/v), was stirred overnight at room temperature. The reaction was quenched by the addition of sat. aqueous NH<sub>4</sub>Cl and extracted with EtOAc. The combined organic layers were washed with water and brine, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo to give the title compound as a colorless solid. PMR (d<sub>6</sub>-DMSO): δ 1.29 (6H, s), 2.42 (2H, d, J = 4.6 Hz), 6.68 (1H, t, J = 4.6 Hz), 7.51 (2H, m), 7.62 (2H, d, J = 8.2 Hz), 7.77 (1H, d, J = 3.3 Hz), 7.93 (2H, d, J = 8.2 Hz), 7.98 (1H, d, J = 3.3 Hz).

4-[(5,6-Dihydro-5,5-dimethyl-8-(4-methylthiazol-2-yl)-2-naphthalenyl)ethynyl]benzoic acid (Compound 31)

To a solution of ethyl 4-[5,6-dihydro-5,5-dimethyl-8-(4-methylthiazol-2-yl)-2-naphthalenyl]ethynylbenzoate (Compound 16) (145.0 mg, 0.34 mmol) and 4 ml of EtOH at room temperature was added aqueous NaOH (1 ml, 1 M, 1 mmol). The resulting solution was warmed to 50°C for 1 hour and concentrated in vacuo. The residue was suspended in a solution of CH<sub>2</sub>Cl<sub>2</sub> and ether (5:1) and acidified to pH 5 with 1M aqueous HCl. The layers were

1 separated and the organic layer was washed with brine, dried ( $\text{Na}_2\text{SO}_4$ ),  
2 filtered and the solvents removed under reduced pressure to give the title  
3 compound as a white solid.  $^1\text{H}$  NMR ( $\text{d}_6\text{-DMSO}$ ):  $\delta$  7.94 (2H, d,  $J$  = 8.1  
4 Hz), 7.87 (1H, d,  $J$  = 1.6 Hz), 7.63 (2H, d,  $J$  = 8.3 Hz), 7.50 (1H, dd,  $J$  =  
5 1.6, 8.1 Hz), 7.45 (1H, d,  $J$  = 8.1 Hz), 7.27 (1H, s), 6.58 (1H, t,  $J$  = 4.8 Hz),  
6 2.43 (3H, s), 2.37 (2H, d,  $J$  = 4.8 Hz), 1.26 (6H, s).

7 4-[(5,6-Dihydro-5,5-dimethyl-8-(4,5-dimethylthiazol-2-yl))-2-  
8 naphthalenyl]ethynyl] benzoic acid (Compound 32)

9 To a solution of ethyl 4-[5,6-dihydro-5,5-dimethyl-8-(4,5-  
10 dimethylthiazol-2-yl)-2-naphthalenyl]ethynylbenzoate (Compound 17) (58.0  
11 mg, 0.13 mmol) and 4 ml of EtOH at room temperature was added aqueous  
12 NaOH (1 ml, 1 M, 1 mmol). The resulting solution was warmed to  $50^\circ\text{C}$  for 1  
13 hour and concentrated in vacuo. The residue was suspended in a solution of  
14  $\text{CH}_2\text{Cl}_2$  and ether (5:1) and acidified to pH 5 with 1M aqueous HCl. The  
15 layers were separated and the organic layer was washed with brine, dried  
16 ( $\text{Na}_2\text{SO}_4$ ), filtered and the solvents removed under reduced pressure to give  
17 the title compound as a white solid.  $^1\text{H}$  NMR ( $\text{d}_6\text{-DMSO}$ ):  $\delta$  7.94 (2H, d,  $J$   
18 = 8.4 Hz), 7.86 (1H, d,  $J$  = 1.6 Hz), 7.61 (2H, d,  $J$  = 8.3 Hz), 7.50 (1H, dd,  $J$   
19 = 1.6, 8.0 Hz), 7.45 (1H, d,  $J$  = 8.0 Hz), 6.51 (1H, t,  $J$  = 4.9 Hz), 2.37 (3H,  
20 s), 2.36 (2H, d,  $J$  = 4.6 Hz), 2.32 (3H, s), 1.26 (6H, s).

21 Ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(5-methyl-2-thienyl))-2-naphthalenyl]  
22 ethynyl] benzoate (Compound 33)

23 Employing the same general procedure as for the preparation of ethyl  
24 4-[(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl))-2-  
25 naphthalenyl]ethynyl]benzoate (Compound 1), 170.0 mg (0.366 mmol) of  
26 ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(trifluoromethylsulfonyl)oxy)-2-  
27 naphthalenyl]ethynyl]benzoate (Compound G) was converted into the title  
28 compound (colorless solid) using 202.0 mg (1.48 mmol) of zinc chloride, 24  
29 mg (0.022 mmol) of tetrakis(triphenylphosphine)palladium(0) in 2.0 ml of



1 THF, and 5-methyl-2-lithiothiophene (prepared by adding 58.6 mg (0.36 ml,  
2 0.915 mmol) of n- butyllithium (2.5 M solution in hexanes) to a cold solution  
3 (-78°C) of 89.8 mg (0.915 mmol) of 2-methylthiophene in 2.0 ml of THF).  
4 <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 8.00 (2H, d, J = 8.3 Hz), 7.59 (1H, d, J = 1.7 Hz), 7.55  
5 (2H, d, J = 8.2 Hz), 7.43 (1H, dd, J = 1.7, 8.0 Hz), 7.35 (1H, d, J = 8.0 Hz),  
6 6.87 (1H, d, J = 3.5 Hz), 6.74 (1H, d, J = 2.8 Hz), 6.15 (1H, t, J = 4.8 Hz),  
7 4.38 (2H, q, J = 7.1 Hz), 2.52 (3H, s), 2.32 (2H, d, J = 4.8 Hz). 1.40 (3H, t,  
8 7.1 Hz), 1.32 (6H, s).

9 Ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(2-thienyl)-2-  
10 naphthalenyl)ethynyl]benzoate (Compound 33a)

11 Employing the same general procedure as for the preparation of ethyl  
12 4-[(5,6-dihydro-5,5-dimethyl-8-(4- methylphenyl)-2-  
13 naphthalenyl)ethynyl]benzoate (Compound 1), 250.0 mg (0.52 mmol) of ethyl  
14 4-[(5,6-dihydro-5,5- dimethyl-8-(trifluoromethylsulfonyl)oxy-2-  
15 naphthalenyl)ethynyl]benzoate (Compound G) was converted into the title  
16 compound (colorless solid) using 186.8 mg (1.37 mmol) of zinc chloride 37.1  
17 mg (0.03 mmol) of tetrakis(triphenylphosphine)palladium(0) and 2-  
18 lithiothiophene (prepared by the addition of 65.9 mg (0.69 ml, 1.03 mmol) of  
19 n-butyllithium (1.5M solution in hexane) to a cold solution (-78 oC) of 86.5  
20 mg (1.03 mmol) of thiophene in 1.0 ml of THF). PMR (CDCl<sub>3</sub>): δ 1.33 (6H,  
21 s), 1.36 (3H, t, J = 7.1 Hz), 2.38 (2H, d, J = 4.7 Hz), 4.34 (2H, q, J = 7.2  
22 Hz), 6.25 (1H, t, J = 4.7 Hz), 7.13 ( 2H, m), 7.47 (4H, m), 7.62 ( 2H, d, J =  
23 8.5 Hz), 8.00 (2H, d, J = 8.5 Hz).

24 4-[(5,6-Dihydro-5,5-dimethyl-8-(5-methyl-2-thienyl)-2-naphthalenyl)ethynyl]  
25 benzoic acid (Compound 34)

26 To a solution of ethyl 4-[5,6-dihydro-5,5-dimethyl-8-(5-methyl-2-  
27 thienyl)-2-naphthalenyl]ethynylbenzoate (Compound 33) (35.0 mg, 0.082  
28 mmol) in 2 ml of EtOH and 1 ml THF at room temperature was added  
29 aqueous NaOH (1 ml, 1 M, 1 mmol). The resulting solution was stirred at

1 room temperature overnight and then acidified with 10% HCl. Extraction  
2 with EtOAc, followed by drying over  $\text{Na}_2\text{SO}_4$ , and removal of the solvents  
3 under reduced pressure afforded the title compound as a colorless solid.  $^1\text{H}$   
4 NMR ( $\text{d}_6$ -acetone):  $\delta$  8.03 (2H, d,  $J = 8.6$  Hz), 7.63 (2H, d,  $J = 8.6$  Hz),  
5 7.54-7.48 (3H, m), 6.89 (1H, m), 6.18 (1H, t,  $J = 4.7$  Hz), 2.49 (3H, s), 2.35  
6 (2H, d,  $J = 4.7$  Hz), 1.32 (6H, s).

7 4-[(5,6-dihydro-5,5-dimethyl-8-(2-thienyl)-2-naphthalenyl)ethynyl]benzoic acid  
8 (Compound 34a)

9 Employing the same general procedure as for the preparation of 4-  
10 [(5,6-dihydro-5,5-dimethyl-8-(2-thiazolyl)-2-naphthalenyl)ethynyl]benzoic acid  
11 (Compound 30a), 70.0 mg (0.17 mmol) of ethyl 4-[(5,6-dihydro-5,5-  
12 dimethyl-8-(2-thienyl)-2-naphthalenyl)ethynyl]benzoate (Compound 33a) was  
13 converted into the title compound (colorless solid) using 17.8 mg (0.42 mmol)  
14 of LiOH in  $\text{H}_2\text{O}$ . PMR ( $\text{d}_6$ -DMSO):  $\delta$  1.27 (6H, s), 2.33 (2H, d,  $J = 4.9$  Hz),  
15 6.23 (1H, t,  $J = 4.9$  Hz), 7.14 (2H, m), 7.38 - 7.56 (4H, m), 7.61 (2H, d,  $J =$   
16 8.3 Hz), 7.92 (2H, d,  $J = 8.3$  Hz).

17 5,6-Dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-naphthalenecarboxylic acid  
18 (Compound K)

19 A solution of 3,4-dihydro-1-(4-methylphenyl)-4,4-dimethyl-7-  
20 bromonaphthalene (Compound D) (250.0 mg, 0.764 mmol) in 2.0 ml of THF  
21 was cooled to  $-78^\circ\text{C}$  and 1.0 ml of  $t$ -butyllithium (1.68 mmol, 1.7 M solution  
22 in pentane) was added slowly. After stirring for 1 hour at  $-78^\circ\text{C}$  gaseous  $\text{CO}_2$   
23 (generated by evaporation of Dry-Ice, and passed through a drying tube) was  
24 bubbled through the reaction for 1 hour. The solution was then allowed to  
25 warm to room temperature and the reaction was quenched by the addition of  
26 10% HCl. Extraction with EtOAc was followed by washing the combined  
27 organic-layers with  $\text{H}_2\text{O}$  and saturated aqueous NaCl, and drying over  
28  $\text{MgSO}_4$ . Removal of the solvents under reduced pressure and washing of the  
29 solid with hexanes afforded the title compound as a colorless solid.  $^1\text{H}$  NMR

(CDCl<sub>3</sub>):  $\delta$  7.94 (1H, dd, J = 1.8, 8.1 Hz), 7.76 (1H, d, J = 1.8 Hz), 7.45 (1H, d, J = 8.1 Hz), 7.24 (4H, m), 6.01 (1H, t, J = 4.7 Hz), 2.40 (3H, s), 2.36 (2H, d, J = 4.7 Hz), 1.35 (6H, s).

Ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-naphthalenyl)carbonyl]amino]-benzoate (Compound 35)

A solution of 170.0 mg (0.58 mmol) 5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-naphthalenecarboxylic acid (Compound K) 115.0 mg (0.70 mmol) of ethyl 4-aminobenzoate, 145.0 mg (0.76 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, and 92.4 mg (0.76 mmol) of 4-dimethylaminopyridine in 6.0 ml of DMF was stirred overnight at room temperature. Ethyl acetate was added and the resulting solution washed with H<sub>2</sub>O, saturated aqueous NaHCO<sub>3</sub>, and saturated aqueous NaCl, then dried over MgSO<sub>4</sub>. After removal of the solvent under reduced pressure, the product was isolated as a colorless solid by column chromatography (10 to 15% EtOAc/hexanes). <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  8.02 (2H, d, J = 8.7 Hz), 7.72 (2H, m), 7.65 (2H, d, J = 8.7 Hz), 7.52 (1H, d, J = 1.8 Hz), 7.48 (1H, d, J = 8.0 Hz), 7.25 (4H, m), 6.15 (1H, t, J = 4.9 Hz), 4.36 (2H, q, J = 7.1 Hz), 2.40 (3H, s), 2.38 (2H, d, J = 4.9 Hz), 1.39 (3H, t, J = 7.1 Hz), 1.37 (6H, s).

4-[(5,6-Dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-naphthalenyl)carbonyl]amino]-benzoic acid (Compound 36)

To a solution of 26.5 mg (0.06 mmol) ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-naphthalenyl)carbonyl]amino]-benzoate (Compound 35) in 3.0 ml EtOH and 4.0 ml of THF was added 240.1 mg NaOH (6.00 mmol, 3.0 ml of a 2M aqueous solution). After stirring at room temperature for 72 hours, the reaction was quenched by the addition of 10% HCl. Extraction with EtOAc, and drying of the organic layers over MgSO<sub>4</sub>, provided a solid after removal of the solvent under reduced pressure. Crystallization from CH<sub>3</sub>CN afforded the title compound as a colorless solid.

1 <sup>1</sup>H NMR (d<sub>6</sub>-DMSO): δ 10.4 (1H, s), 7.91-7.81 (5H, m), 7.54 (1H, d, J =  
2 8.1 Hz), 7.45 (1H, d, J = 1.7 Hz), 7.23 (4H, s), 6.04 (1H, t, J = 4.7 Hz), 2.35  
3 (5H, s), 1.33 (6H, s).

4 Ethyl 4-[[[(5,6-dihydro-5,5-dimethyl-8-(4-methyl-phenyl)-2-  
5 naphthalenyl)carbonyl]oxy]-benzoate (Compound 37)

6 A solution of 25.0 mg (0.086 mmol) 5,6-dihydro-5,5-dimethyl-8-(4-  
7 methylphenyl)-2-naphthalenecarboxylic acid (Compound K) 17.5 mg (0.103  
8 mmol) of ethyl 4-hydroxybenzoate, 21.4 mg (0.112 mmol) of 1-(3-  
9 dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, and 12.6 mg (0.103  
10 mmol) of 4-dimethylaminopyridine in 2.0 ml of DMF was stirred overnight at  
11 room temperature. Ethyl acetate was added and the resulting solution  
12 washed with H<sub>2</sub>O, saturated aqueous NaHCO<sub>3</sub>, and saturated aqueous NaCl,  
13 before being dried over MgSO<sub>4</sub>. After removal of the solvent under reduced  
14 pressure, the product was isolated by column chromatography as a pale-  
15 yellow solid (10% EtOAc/hexanes). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 8.08 (2H, d, J =  
16 8.1 Hz), 8.05 (1H, dd, J = 1.8, 8.1 Hz), 7.89 (1H, d, J = 1.8 Hz), 7.50 (2H, d,  
17 J = 8.1 Hz), 7.22 (5H, m), 6.05 (1H, t, J = 4.7 Hz), 4.37 (2H, q, J = 7.1 Hz),  
18 2.39 (2H, d, J = 4.7 Hz), 2.38 (3H, s), 1.39 (3H, t, J = 7.1 Hz), 1.37 (6H, s).

19 2-Trimethylsilylethyl 4-[[[(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-  
20 naphthalenyl)carbonyl]oxy]-benzoate (Compound 38)

21 A solution of 93.5 mg (0.320 mmol) 5,6-dihydro-5,5-dimethyl-8-(4-  
22 methylphenyl)-2-naphthalenecarboxylic acid (Compound K) 76.0 mg (0.319  
23 mmol) of 2-trimethylsilylethyl-4-hydroxybenzoate, 80.0 mg (0.417 mmol) of 1-  
24 (3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, and 51.0 mg  
25 (0.417 mmol) of 4-dimethylaminopyridine in 4.0 ml of DMF was stirred  
26 overnight at room temperature. Ethyl acetate was added and the resulting  
27 solution washed with H<sub>2</sub>O, saturated aqueous NaHCO<sub>3</sub>, and saturated  
28 aqueous NaCl, before being dried over MgSO<sub>4</sub>. After removal of the solvent  
29 under reduced pressure, the product was isolated as a colorless solid by

1 column chromatography (5% EtOAc/hexanes).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  8.08  
2 (2H, d,  $J = 8.8$  Hz), 8.05 (1H, dd,  $J = 1.8, 8.1$  Hz), 7.50 (1H, d,  $J = 8.1$  Hz),  
3 7.26- 7.18 (6H, m), 6.05 (1H, t,  $J = 4.7$  Hz), 4.42 (2H, t,  $J = 8.4$  Hz), 2.40  
4 (2H, d,  $J = 4.7$  Hz), 2.39 (3H, s), 1.38 (6H, s), 0.09 (9H, s).

5 4-[(5,6-Dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-  
6 naphthalenyl)carbonyl]oxy]-benzoic acid (Compound 39)

7 A solution of 110.0 mg (0.213 mmol) 2- trimethylsilylethyl 4[(5,6-  
8 dihydro-5,5-dimethyl-8-(4- methylphenyl)-2-naphthalenyl)carbonyl]oxy]-  
9 benzoate (Compound 38) and 167.3 mg of tetrabutylammonium flouride  
10 (0.640 mmol, 0.64 ml of a 1M solution in THF) in 2.0 ml THF was stirred at  
11 room temperature for 22 hours. Ethyl acetate was added and the resulting  
12 solution washed with  $\text{H}_2\text{O}$  and saturated aqueous NaCl then dried over  
13  $\text{MgSO}_4$ . Removal of the solvents under reduced pressure and washing of the  
14 residual solid with EtOAc and  $\text{CH}_3\text{CN}$  afforded the title compound as a  
15 colorless solid.  $^1\text{H}$  NMR ( $d_6$ -acetone):  $\delta$  8.10 (2H, d,  $J = 8.8$  Hz), 8.06 (1H,  
16 dd,  $J = 2.0, 8.1$  Hz), 7.82 (1H, d,  $J = 1.9$  Hz), 7.64 (1H, d,  $J = 8.1$  Hz), 7.35  
17 (2H, d,  $J = 8.6$  Hz), 7.25 (4H, m), 6.08 (1H, t,  $J = 4.7$  Hz), 2.42 (2H, d,  $J =$   
18 4.7 Hz), 2.35 (3H, s), 1.39 (6H, s).

19 Ethyl 2-fluoro-4-[(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-  
20 naphthalenyl)carbonyl]amino]-benzoate (Compound 40)

21 A solution of 115.0 mg (0.41 mmol) 5,6-dihydro-5,5-dimethyl-8-(4-  
22 methylphenyl)-2-naphthalenecarboxylic acid (Compound K) 89.0 mg (0.49  
23 mmol) of ethyl 2-fluoro-4-aminobenzoate, 102.0 mg (0.53 mmol) of 1-(3-  
24 dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, and 65.0 mg (0.53  
25 mmol) of 4-dimethylaminopyridine in 5.0 ml of DMF was stirred at  $50^\circ\text{C}$  for  
26 1 hour and then overnight at room temperature. Ethyl acetate was added  
27 and the resulting solution washed with  $\text{H}_2\text{O}$ , saturated aqueous  $\text{NaHCO}_3$ , and  
28 saturated aqueous NaCl, before being dried over  $\text{MgSO}_4$ . After removal of  
29 the solvent under reduced pressure, the product was isolated as a colorless

1 solid by column chromatography (20% EtOAc/hexanes). <sup>1</sup>H NMR (CDCl<sub>3</sub>):  
2 δ 7.96 (1H, s), 7.89 (1H, t, J = 8.4 Hz), 7.70 (2H, m), 7.52 (1H, d, J = 1.9  
3 Hz), 7.45 (1H, d, J = 8.1 Hz), 7.23 (5H, m), 6.04 (1H, t, J = 4.8 Hz), 4.36  
4 (2H, q, J = 7.1 Hz), 2.38 (3H, s), 2.35 (2H, d, J = 4.8 Hz), 1.39 (3H, t, J =  
5 7.1 Hz), 1.36 (6H, s).

6 2-Fluoro-4-[(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-  
7 naphthalenyl)carbonyl]amino]-benzoic acid (Compound 41)

8 To a solution of 41.6 mg (0.091 mmol) ethyl 2- fluoro-4-[(5,6-  
9 dihydro-5,5-dimethyl-8- (4-methylphenyl)-2-naphthalenyl)carbonyl]amino]-  
10 benzoate (Compound 40) in 2.0 ml EtOH and 2.0 ml of THF was added 40.0  
11 mg NaOH (1.00 mmol, 1.0 ml of a 1 M aqueous solution). After stirring at  
12 room temperature for overnight, the reaction was quenched by the addition  
13 of 10% HCl. Extraction with EtOAc, and drying of the organic layers over  
14 MgSO<sub>4</sub>, provided a solid after removal of the solvent under reduced pressure.  
15 Crystallization from CH<sub>3</sub>CN afforded the title compound as a pale-yellow  
16 solid. <sup>1</sup>H NMR (d<sub>6</sub>-acetone): δ 9.84 (1H, s), 7.94-7.83 (3H, m), 7.64 (1H,  
17 dd, J = 2.0 Hz), 7.53 (2H, d, J = 8.1 Hz), 7.23 (4H, s), 6.04 (1H, t, J = 4.7  
18 Hz), 2.38 (2H, d, J = 4.7 Hz), 2.36 (3H, s), 1.35 (6H, s).

19 Ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)- 2-  
20 naphthalenyl)thiocarbonyl]amino]-benzoate (Compound 42)

21 A solution of 110.0 mg (0.25 mmol) ethyl 4-[(5,6-dihydro-5,5-  
22 dimethyl-8-(4-methylphenyl)- 2-naphthalenyl)carbonyl]amino]-benzoate  
23 (Compound 35) and 121.0 mg (0.30 mmol) of [2,4-bis(4-methoxyphenyl)- 1,3-  
24 dithia-2,4-diphosphetane-2,4-disulfide] (Lawesson's Reagent) in 12.0 ml of  
25 benzene was refluxed overnight. Upon cooling to room temperature, the  
26 mixture was filtered and the filtrate concentrated under reduced pressure.  
27 The title compound was isolated by column chromatography (10 to 25%  
28 EtOAc / hexanes) as a yellow solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 8.92 (1H, s), 8.06  
29 (2H, t, J = 8.5 Hz), 7.88-7.70 (3H, m), 7.42 (2H, d, J = 8.1 Hz), 7.18 (4H,

1 m), 6.03 (1H, t, J = 4.7 Hz), 4.37 (2H, q, J = 7.1 Hz), 2.38 (3H, s), 2.36 (2H,  
2 d, J = 4.7 Hz), 1.56 (3H, t, J = 7.1 Hz), 1.35 (6H, s).

3 4-[[[(5,6-Dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-  
4 naphthalenyl)thiocarbonyl]amino]-benzoic acid (Compound 43)

5 To a solution of 84.0 mg (0.184 mmol) ethyl 4[[[(5,6-dihydro-5,5-  
6 dimethyl-8-(4-methylphenyl)-2- naphthalenyl)thiocarbonyl]amino]-benzoate  
7 (Compound 42) in 2.0 ml EtOH and 2.0 ml of THF was added 60.0 mg  
8 NaOH (1.50 mmol, 1.5 ml of a 1 M aqueous solution). After stirring at room  
9 temperature overnight, the reaction was quenched by the addition of 10%  
10 HCl. Extraction with EtOAc, and drying of the organic layers over MgSO<sub>4</sub>,  
11 provided a solid after removal of the solvent under reduced pressure.  
12 Crystallization from CH<sub>3</sub>CN afforded the title compound as a yellow solid.  
13 <sup>1</sup>H NMR (d<sub>6</sub>-acetone): δ 10.96 (1H, s), 8.05 (4H, m), 7.72 (1H, dd, J = 2.0,  
14 8.0 Hz), 7.54 (1H, s), 7.46 (1H, d, J = 8.1 Hz), 7.20 (4H, m), 6.04 (1H, t, J =  
15 4.7 Hz), 2.38 (2H, d, J = 4.7 Hz), 2.33 (3H, s), 1.35 (6H, s).

16 2-acetyl-6-bromonaphthalene (Compound L)

17 To a cold (10°C) mixture of 44.0 g (0.212 mol) of 2-bromonaphthalene  
18 and 34.0 g (0.255 mol) of aluminum chloride in 400 ml of nitrobenzene was  
19 added 21.0 g (267 mmol) of acetyl chloride. The mechanically stirred  
20 reaction mixture was warmed to room temperature, and heated to 40°C for  
21 18 hours. After cooling to 0°C in an ice bath, the reaction was quenched by  
22 the addition of 12M HCl (70 ml). The layers were separated and the organic  
23 phase was washed with water and dilute aqueous Na<sub>2</sub>CO<sub>3</sub>. Kugelrohr  
24 distillation, followed by recrystallization from 10% EtOAc-hexane yielded 23  
25 g of the title compound as a tan solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 8.44 (1H, br s),  
26 8.04-8.10 (2H, m), 7.85 (1H, d, J = 8.5 Hz), 7.82 (1H, d, J = 8.8 Hz), 7.64  
27 (1H, d, J = 8.8 Hz), 2.73 (3H, s).

28 6-bromo-2-naphthalenecarboxylic acid (Compound M)

29 To a solution of sodium hypochlorite (62 ml, 5.25% in water (w/w), 3.6

1 g, 48.18 mmol) and sodium hydroxide (6.4 g, 160.6 mmol) in 50 ml of water  
2 was added a solution of 2-acetyl-6-bromonaphthalene (Compound L) 4 g,  
3 (16.06 mmol) in 50 ml of 1,4-dioxane. The yellow solution was heated to  
4 70°C in an oil bath for 2 hours, cooled to ambient temperature, and extracted  
5 with ethyl ether (2 x 50 ml). The aqueous layers were diluted with NaHSO<sub>3</sub>  
6 solution (until KI indicator solution remained colorless) and then acidified  
7 (pH <2) with 1N sulfuric acid to give a white precipitate. The mixture was  
8 extracted with ethyl ether, and the combined organic phase washed with  
9 saturated aqueous NaCl, dried (MgSO<sub>4</sub>) and concentrated to give 3.54 g  
10 (88%) of the title compound as a solid. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 8.63 (1H,  
11 br s), 8.32 (1H, d, J = 2.0 Hz), 8.10 (1H, d, J = 8.8 Hz), 8.00-8.05 (2H, m),  
12 7.74 (1H, dd, J = 2.0, 8.8 Hz).

13 Ethyl 6-bromo-2-naphthalenecarboxylate (Compound N)

14 To a solution of 6-bromo-2-naphthalenecarboxylic acid (Compound M)  
15 3.1 g, (12.43 mmol) in ethanol (30 ml, 23.55 g, 511.0 mmol) was added 18M  
16 sulfuric acid (2 ml). The solution was refluxed for 30 minutes, cooled to  
17 room temperature, and the reaction mixture partitioned between pentane  
18 (100 ml) and water (100 ml). The aqueous phase was extracted with pentane  
19 (100 ml) and the combined organic layers washed with saturated aqueous  
20 NaCl (100 ml), dried (MgSO<sub>4</sub>), and concentrated to yield an off-white solid.  
21 Purification by flash chromatography (silica, 10% EtOAc-hexane) afforded  
22 the title compound as a white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 8.58 (1H, br s),  
23 8.10 (1H, dd, J = 1.7, 9 Hz), 8.06 (1H, d, J = 2 Hz), 7.83 (1H, d, J = 9 Hz),  
24 7.80 (1H, d, J = 9 Hz), 7.62 (1H, dd, J = 2, 9 Hz).

25 Ethyl (E)-4-[2-(5,6,7,8-tetrahydro-5,5-dimethyl-8-oxo-2-naphthalenyl)ethenyl]-  
26 benzoate (Compound O)

27 To a solution of 520.0 mg (2.00 mmol) of 3,4-dihydro-4,4-dimethyl-7-  
28 bromo-1(2H)-naphthalenone (Compound B) and 510.0 mg (2.90 mmol) of  
29 ethyl 4-vinylbenzoate in 4.0 ml of triethylamine (degassed by sparging with



1 argon for 25 minutes), was added 124.0 mg (0.40 mmol) of tris(2-  
2 methylphenyl) phosphine, followed by 44.0 mg (0.20 mmol) of  
3 palladium(II)acetate. The resulting solution was heated to 95°C for 2.5  
4 hours, cooled to room temperature, and concentrated under reduced  
5 pressure. Purification by column chromatography (10% EtOAc / hexanes)  
6 afforded the title compound as a colorless solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 8.19  
7 (1H, d, J = 2.0 Hz), 8.03 (2H, d, J = 8.4 Hz), 7.69 (1H, dd, J = 2.0, 8.2 Hz),  
8 7.57 (2H, d, J = 8.4 Hz), 7.45 (1H, d, J = 8.2 Hz), 7.20 (2H, s), 4.39 (2H, q,  
9 J = 7.1 Hz), 2.76 (2H, t, J = 6.5 Hz), 2.04 (2H, t, J = 6.5 Hz), 1.41(3H, t, J  
10 = 7.1 Hz, and 6H, s).

11 Ethyl (E)-4-[2-(5,6-dihydro-5,5-dimethyl-8-(trifluoromethylsulfonyl)oxy-2-  
12 naphthalenyl)ethenyl]- benzoate (Compound P)

13 To a cold (-78°C) solution of 440.0 mg (2.40 mmol) of sodium  
14 bis(trimethylsilyl)amide in 10.0 ml of THF was added 700.0 mg (2.00 mmol)  
15 of ethyl (E)-4-[2- (5,6,7,8-tetrahydro-5,5-dimethyl-8-oxo-2-  
16 naphthalenyl)ethenyl]-benzoate (Compound O) as a solution in 25.0 ml of  
17 THF. After stirring at -78°C for 1.5 hours, 960.0 mg (2.40 mmol) of 2[N,N-  
18 bis(trifluoromethylsulfonyl)amino]-5-chloropyridine was added in one portion.  
19 After 30 minutes the solution was warmed to 0°C and stirred for 3 hours.  
20 The reaction was quenched by the addition of saturated aqueous NH<sub>4</sub>Cl, and  
21 extracted with EtOAc. The combined extracts were washed with 5% aqueous  
22 NaOH, dried (Na<sub>2</sub>SO<sub>4</sub>), and the solvents removed under reduced pressure.  
23 The title compound was isolated as a colorless solid by column  
24 chromatography (7% EtOAc / hexanes). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 8.04 (1H, d, J  
25 = 8.4 Hz), 7.57 (2H, d, J = 8.4 Hz), 7.52 (1H, s), 7.49 (1H, d, J = 8.0 Hz),  
26 7.33 (1H, d, J = 8.0 Hz), 7.20 (1H, d, J = 16.4 Hz), 7.10 (1H, d, J = 16.4  
27 Hz), 6.00 (1H, t, J = 4.9 Hz), 4.39 (2H, q, J = 7.1 Hz), 2.43 (2H, d, J = 4.9  
28 Hz), 1.41 (3H, t, J = 7.1 Hz), 1.32 (6H, s).

29 Ethyl (E)-4-[2-(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-

1 naphthalenyl)ethenyl]-benzoate (Compound 44)

2 A solution of 4-lithiotoluene was prepared at  $-78^{\circ}\text{C}$  by the addition of  
3 130.7 mg of t-butyllithium (2.04 mmol; 1.20 ml of a 1.7M solution in pentane)  
4 to a solution of 374.5 mg (2.20 mmol) of 4-bromotoluene in 2.5 ml of THF.  
5 After 30 minutes a solution of 313.4 mg (2.30 mmol) of  $\text{ZnCl}_2$  in 2.0 ml of  
6 THF was added. The resulting solution was warmed to room temperature,  
7 stirred for 1.25 hour and then added via canula to a solution of 285.0 mg  
8 (0.590 mmol) of ethyl (E)-4-[2-(5,6-dihydro-5,5-dimethyl-8-  
9 (trifluoromethylsulfonyl)oxy-2-naphthalenyl)ethenyl]-benzoate (Compound P)  
10 and 29.0 mg (0.025 mmol) of tetrakis(triphenylphosphine)palladium(0) in 2.0  
11 ml of THF. The resulting solution was stirred at room temperature for 1  
12 hour and then at  $55^{\circ}\text{C}$  for 2 hours. Upon cooling to room temperature the  
13 reaction was quenched by the addition of saturated aqueous  $\text{NH}_4\text{Cl}$ . The  
14 mixture was extracted with EtOAc, and the combined extracts were washed  
15 with 5% aqueous NaOH, saturated aqueous NaCl, and dried over  $\text{Na}_2\text{SO}_4$   
16 before being concentrated under reduced pressure. The title compound was  
17 isolated by column chromatography (10% EtOAc / hexanes) as a colorless  
18 solid.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  7.96 (2H, d,  $J = 8.1$  Hz), 7.47 (2H, d,  $J = 8.1$   
19 Hz), 7.43-7.16 (7H, m), 7.07 (1H, d,  $J = 16.3$  Hz), 6.93 (1H, d,  $J = 16.3$  Hz),  
20 5.97 (1H, t,  $J = 4.7$  Hz), 4.39 (2H, q,  $J = 7.0$  Hz), 2.41 (3H, s), 2.33 (1H, d,  $J$   
21 = 4.7 Hz), 1.38 (3H, t,  $J = 7.0$  Hz), 1.33 (6H, s).

22 (E)-4-[2-(5,6-Dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-  
23 naphthalenyl)ethenyl]-benzoic acid Compound 45

24 To a solution of 65.0 mg (0.190 mmol) of ethyl (E)-4-[2-(5,6-  
25 dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-naphthalenyl)ethenyl]-benzoate  
26 (Compound 44) in 4.0 ml of THF was added 30.0 mg of LiOH (0.909 mmol,  
27 1.0 ml of a 1.1M solution) and 1.0 ml of MeOH. The solution was heated to  
28  $55^{\circ}\text{C}$  for 3 hours, cooled to room temperature, and concentrated under  
29 reduced pressure. The residue was dissolved in  $\text{H}_2\text{O}$  and extracted with

1 hexanes. The aqueous layer was acidified to pH 1 with 10% HCl, and  
2 extracted with Et<sub>2</sub>O. The combined organic layers were washed with  
3 saturated aqueous NaCl, diluted with EtOAc to give a clear solution, and  
4 dried over Na<sub>2</sub>SO<sub>4</sub>. The solvents were removed under reduced pressure to  
5 give the title compound as a colorless solid. <sup>1</sup>H NMR (d<sub>6</sub>-DMSO): δ 7.86  
6 (2H, d, J = 8.4 Hz), 7.66 (2H, d, J = 8.4 Hz), 7.58 (1H, dd, J = 1.7, 8.1 Hz),  
7 7.41 (1H, d, J = 8.1 Hz), 7.28 (1H, d, J = 16.5 Hz), 7.23 (4H, s), 7.08 (1H, d,  
8 J = 1.7 Hz), 7.07 (1H, d, J = 16.5 Hz), 5.97 (1H, t, J = 4.6 Hz), 2.35 (3H, s),  
9 2.31 (1H, d, J = 4.6 Hz), 1.29 (6H, s).

10 Ethyl 4-[2-(1,1-dimethyl-3-(4-methylphenyl)-5-indenyl)ethynyl]benzoate  
11 (Compound 47)

12 A solution of 32.0 mg (0.187 mmol) of 4-bromotoluene in 1.0 ml THF  
13 was cooled to -78°C and 24.0 mg of t-butyllithium (0.375 mmol, 0.22 ml of a  
14 1.7 M solution in pentane) was slowly added. The yellow solution was stirred  
15 for 30 minutes at which time 29.8 mg (0.219 mmol) of ZnCl<sub>2</sub> was added as a  
16 solution in 1.0 ml THF. The resulting solution was warmed to room  
17 temperature and after 30 minutes added to a second flask containing 29.0 mg  
18 (0.062 mmol) of ethyl 4-[2-(1,1-dimethyl-3-(trifluoromethylsulfonyl)oxy-5-  
19 indenyl)ethynyl]benzoate (Compound FF) and 2.9 mg (0.003 mmol) of  
20 tetrakis(triphenylphosphine)palladium (0) in 1.0 ml THF. The resulting  
21 solution was warmed to 50°C for 1 hour and then stirred at room  
22 temperature for 4 hours. The reaction was quenched by the addition of  
23 saturated aqueous NH<sub>4</sub>Cl, and then extracted with Et<sub>2</sub>O. The combined  
24 organic layers were washed with water, saturated aqueous NaCl, and dried  
25 over MgSO<sub>4</sub> before being concentrated under reduced pressure. The title  
26 compound was isolated as a colorless oil by column chromatography (10%  
27 Et<sub>2</sub>O/-hexanes). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 8.03 (2H, d, J = 8.5 Hz),  
28 7.66 (1H, s), 7.58 (2H, d, J = 8.5 Hz), 7.50 (2H, d, J = 8.0 Hz), 7.46 (1H, d,  
29 J = 7.9 Hz), 7.38 (1H, d, J = 7.7 Hz), 7.28 (2H, d, J = 9 Hz), 6.43 (1H, s),

1 4.40 (2H, q, J = 7.2 Hz), 2.43 (3H, s), 1.41 (3H, t; + 6H, s).

2 4-[2-(1,1-dimethyl-3-(4-methylphenyl)-5-indenyl)ethynyl]benzoic acid

3 (Compound 48)

4 To a solution of 10.0 mg (0.025 mmol) of ethyl 4-[2-(1,1-dimethyl-3-  
5 (4-methylphenyl)-5-indenyl)ethynyl]benzoate (Compound 47) in 0.5 ml  
6 THF/H<sub>2</sub>O (3:1 v/v) was added 5.2 mg (0.12 mmol) LiOH H<sub>2</sub>O. After stirring  
7 at room temperature for 48 hours the solution was extracted with hexanes  
8 and the aqueous layer was acidified with saturated aqueous NH<sub>4</sub>Cl. Solid  
9 NaCl was added and the resulting mixture extracted with EtOAc. The  
10 combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated under  
11 reduced pressure to give the title compound as a colorless solid. <sup>1</sup>H NMR  
12 (300 MHz, d<sub>6</sub>-DMSO): δ 7.95 (2H, d, J = 8.3 Hz), 7.65 (2H, d, J = 8.3 Hz),  
13 7.57 (2H, m), 7.49 (3H, m), 7.30 (2H, d, J = 7.9 Hz), 6.61 (1H, s), 2.36 (3H,  
14 s), 1.36 (6H, s).

15 3-(4-bromothiophenoxy)propionic acid

16 To a solution of 1.44 g (35.7 mmol) of NaOH in 20.0 ml degassed H<sub>2</sub>O  
17 (sparged with argon) was added 6.79 g (35.7 mmol) of 4-bromothiophenol.  
18 The resulting mixture was stirred at room temperature for 30 minutes. A  
19 second flask was charged with 2.26 g (16.3 mmol) of K<sub>2</sub>CO<sub>3</sub> and 15 ml of  
20 degassed H<sub>2</sub>O. To this solution was added (in portions) 5.00 g (32.7 mmol) of  
21 3-bromopropionic acid. The resulting potassium carboxylate solution was  
22 added to the sodium thiolate solution, and the resulting mixture stirred at  
23 room temperature for 48 hours. The mixture was filtered and the filtrate  
24 extracted with benzene, and the combined organic layers were discarded. The  
25 aqueous layer was acidified with 10% HCl and extracted with EtOAc. The  
26 combined organic layers were washed with saturated aqueous NaCl, dried  
27 over MgSO<sub>4</sub>, and concentrated under reduced pressure. The resulting solid  
28 was recrystallized from Et<sub>2</sub>O - hexanes to give the title compound as off-  
29 white crystals. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.43 (2H, d, J = 8.4 Hz), 7.25 (2H, d, J

1 = 8.4 Hz), 3.15 (2H, t, J = 7.3 Hz), 2.68 (2H, t, J = 7.3 Hz).

2 2,3-dihydro-6-bromo-(4H)-1-benzothiopyran-4-one

3 A solution of 3.63 g (13.9 mmol) of 3-(4-bromothiophenoxy)propionic  
4 acid in 60 ml methanesulfonic acid was heated to 75°C for 1.5 hours. After  
5 cooling to room temperature the solution was diluted with H<sub>2</sub>O and extracted  
6 with EtOAc. The combined organic layers were washed with 2N aqueous  
7 NaOH, H<sub>2</sub>O, and saturated aqueous NaCl and then dried over MgSO<sub>4</sub>.  
8 Removal of the solvent under reduced pressure afforded a yellow solid from  
9 which the product was isolated by column chromatography (3% EtOAc-  
10 hexanes) as a pale-yellow solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 8.22 (1H, d, J = 2.1  
11 Hz), 7.48 (1H, dd, J = 2.1, 8.3 Hz), 7.17 (1H, d, J = 8.5 Hz), 3.24 (2H, t, J =  
12 6.4 Hz), 2.98 (2H, t, J = 6.7 Hz).

13 2,3-dihydro-6-(2-trimethylsilylethynyl)-(4H)-1-benzothiopyran-4-one

14 A solution of 1.00 g (4.11 mmol) 2,3-dihydro-6-bromo-(4H)-1-  
15 benzothiopyran-4-one and 78.3 mg (0.41 mmol) CuI in 15.0 ml THF and 6.0  
16 ml Et<sub>3</sub>NH was sparged with argon for 5 minutes. To this solution was added  
17 2.0 ml (1.39 g, 14.2 mmol) of (trimethylsilyl)acetylene followed by 288.5 mg  
18 (0.41 mmol) of bis(triphenylphosphine)palladium(II) chloride. The resulting  
19 dark solution was stirred at room temperature for 3 days and then filtered  
20 through a pad of Celite, which was washed with EtOAc. The filtrate was  
21 washed with H<sub>2</sub>O and saturated aqueous NaCl before being dried over  
22 MgSO<sub>4</sub>. The title compound was isolated as an orange oil by column  
23 chromatography (4% EtOAc - hexanes). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 8.13 (1H, d, J  
24 = 1.9 Hz), 7.36 (1H, dd, J = 2.1, 8.2 Hz), 7.14 (1H, d, J = 8.2 Hz), 3.19 (2H,  
25 d, J = 6.3 Hz), 2.91 (2H, d, J = 6.3 Hz), 0.21 (9H, s).

26 2,3-dihydro-6-ethynyl-(4H)-1-benzothiopyran-4-one

27 A solution containing 600.0 mg (2.25 mmol) of 2,3-dihydro-6-(2-  
28 trimethylsilylethynyl)-(4H)-1-benzothiopyran-4-one and 100.0 mg (0.72  
29 mmol) K<sub>2</sub>CO<sub>3</sub> in 15 ml MeOH was stirred at room temperature for 20 hours.

1 The solution was diluted with H<sub>2</sub>O and extracted with Et<sub>2</sub>O. The combined  
2 organic layers were washed with H<sub>2</sub>O and saturated aqueous NaCl before  
3 being dried over MgSO<sub>4</sub>. Removal of the solvents under reduced pressure  
4 afforded the title compound as an orange solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 8.17  
5 (1H, d, J = 1.8 Hz), 7.40 (1H, dd, J = 1.8, 8.2 Hz), 7.19 (1H, d, J = 8.2 Hz),  
6 3.22 (2H, t, J = 6.3 Hz), 3.08 (1H, s), 2.94 (2H, t, J = 6.3 Hz).

7 Ethyl 4-[2-(6-(2,3-dihydro-(4H)-1-benzothiopyran-4-onyl))ethynyl]benzoate

8 A solution of 405.0 mg (2.15 mmol) 2,3-dihydro-6-ethynyl-(4H)-1-  
9 benzothiopyran-4-one and 594.0 mg (2.15 mmol) of ethyl 4-iodobenzoate in  
10 15 ml Et<sub>3</sub>N and 3 ml THF was sparged with argon for 15 minutes. To this  
11 solution was added 503.0 mg (0.72 mmol) of  
12 bis(triphenylphosphine)palladium(II) chloride and 137.0 mg (0.72 mmol) CuI.  
13 This solution was stirred for 20 hours at room temperature and then filtered  
14 through a pad of Celite, which was washed with EtOAc. Removal of the  
15 solvents under reduced pressure afforded a brown solid. Column  
16 chromatography (3% EtOAc-hexanes) afforded the title compound as an  
17 orange solid. <sup>1</sup>H NMR (d<sub>6</sub>-acetone): δ 8.15 (1H, d, J = 2.0 Hz), 8.02 (2H,  
18 d, J = 8.5 Hz), 7.69 (2H, d, J = 8.5 Hz), 7.61 (1H, dd, J = 2.1, 8.3 Hz), 7.40  
19 (1H, d, J = 8.2 Hz), 4.35 (2H, q, J = 7.1 Hz), 3.40 (2H, t, J = 6.3 Hz), 2.96  
20 (2H, t, J = 6.3 Hz), 1.37 (3H, t, J = 7.1 Hz).

21 Ethyl 4-[2-(6-(4-(trifluoromethylsulfonyl)oxy-(2H)-1-benzothiopyra  
22 nyl))ethynyl]benzoate

23 To a solution of 221.9 mg (1.21 mmol) of sodium  
24 bis(trimethylsilyl)amide in 3.0 ml THF cooled to -78°C was added 370.0 mg  
25 (1.10 mmol) of ethyl 4-[2-(6-(2,3-dihydro-(4H)-1-benzothiopyran-4-  
26 onyl))ethynyl]benzoate in 4.0 ml THF. After 30 minutes, a solution of 2-  
27 [N,N-bis(trifluoromethylsulfonyl)amino]-5-chloropyridine in 4.0 ml THF was  
28 slowly added. The reaction was slowly warmed to room temperature and  
29 after 5 hours quenched by the addition of saturated aqueous NH<sub>4</sub>Cl. The

1 mixture was extracted with EtOAc, and the combined organic layers were  
2 washed with 5% aqueous NaOH, H<sub>2</sub>O, and saturated aqueous NaCl before  
3 being dried over MgSO<sub>4</sub>. Removal of the solvents under reduced pressure,  
4 followed by column chromatography (4% EtOAc-hexanes) afforded the title  
5 compound as a pale-yellow solid. <sup>1</sup>H NMR (d<sub>6</sub>-acetone): δ 8.12 (2H, d, J =  
6 8.5 Hz), 7.66 (2H, d, J = 8.5 Hz), 7.56 (1H, d, J = 1.7 Hz), 7.49 (1H, dd, J =  
7 1.7, 8.1 Hz), 7.40 (1H, d, J = 8.1 Hz), 6.33 (1H, t, J = 5.7 Hz), 4.35 (2H, q, J  
8 = 7.1 Hz), 3.82 (2H, d, J = 5.7 Hz), 1.37 (3H, t, J = 7.1 Hz).

9 Ethyl 4-[2-(6-(4-(4-methylphenyl)-(2H)-1-benzothiopyranyl))ethynyl]benzoate  
10 (Compound 49)

11 To a solution of 120.8 mg (0.70 mmol) of 4-bromotoluene in 2.0 ml  
12 THF at -78°C was added 88.4 mg (1.38 mmol, 0.81 ml of a 1.7 M solution in  
13 pentane) of t-butyllithium. After 30 minutes a solution of 131.6 mg (0.97  
14 mmol) ZnCl<sub>2</sub> in 2.0 ml THF was added and the resulting pale-yellow solution  
15 warmed to room temperature. Stirring for 40 minutes was followed by  
16 addition of this solution to a second flask containing 129.2 mg (0.28 mmol) of  
17 ethyl 4-[2-(6-(4-(trifluoromethylsulfonyl)oxy-(2H)-1-  
18 benzothiopyranyl))ethynyl]benzoate, 14.0 mg (0.012 mmol)  
19 tetrakis(triphenylphosphine)palladium (0), and 2.0 ml THF. The resulting  
20 solution was heated to 50°C for 5 hours, cooled to room temperature, and  
21 quenched by the addition of saturated aqueous NH<sub>4</sub>Cl. The mixture was  
22 extracted with EtOAc, and the combined organic layers were washed with  
23 H<sub>2</sub>O and saturated aqueous NaCl, then dried (MgSO<sub>4</sub>) and concentrated to  
24 an orange oil. The title compound was isolated as a colorless solid by  
25 column chromatography (3 to 5% EtOAc-hexanes). <sup>1</sup>H NMR (d<sub>6</sub>-acetone): δ  
26 7.98 (2H, d, J = 8.3 Hz), 7.58 (2H, d, J = 8.2 Hz), 7.44-7.38 (2H, m),  
27 7.26-7.15 (5H, m), 6.14 (1H, t, J = 5.8 Hz), 4.34 (2H, q, J = 7.1 Hz), 3.53  
28 (2H, d, J = 5.8 Hz), 2.37 (2H, s), 1.35 (3H, t, J = 7.1 Hz).  
29 4-[2-(6-(4-(4-methylphenyl)-(2H)-1-benzothiopyranyl))ethynyl]-benzoic acid

1 (Compound 50)

2 To a solution of 29.0 mg (0.07 mmol) ethyl 4-[2-(6-(4-(4-  
3 methylphenyl)-(2H)-1-benzothiopyranyl))ethynyl]benzoate (Compound 49)  
4 in 2.0 ml THF and 2.0 ml EtOH was added 160.0 mg (4.00 mmol, 2.0 ml of a  
5 2 M aqueous solution). The resulting solution was stirred at 35°C for 2  
6 hours, and then cooled to room temperature and stirred an additional 2  
7 hours. The reaction was quenched by the addition of 10% aqueous HCl and  
8 extracted with EtOAc. The combined organic layers were washed with H<sub>2</sub>O  
9 and saturated aqueous NaCl, and dried over Na<sub>2</sub>SO<sub>4</sub>. Removal of the  
10 solvents under reduced pressure afforded a solid which was washed with  
11 CH<sub>3</sub>CN and dried under high vacuum to give the title compound as a pale-  
12 yellow solid. <sup>1</sup>H NMR (d<sub>6</sub>-DMSO): δ 7.90 (2H, d, J = 8.4 Hz), 7.59 (2H, d,  
13 J = 8.4 Hz), 7.40 (4H, m), 7.25-7.13 (4H, m), 7.02 (1H, d, J = 1.7 Hz), 6.11  
14 (1H, t, J = 5.7 Hz), 3.54 (2H, d, J = 5.7 Hz), 2.34 (3H, s).

15 3,4-Dihydro-4,4-dimethyl-7-acetyl-1(2H)-naphthalenone (Compound R); and  
16 3,4-dihydro-4,4-dimethyl-6-acetyl-1(2H)-naphthalenone (Compound S)

17 To a cold (0°C) mixture of aluminum chloride (26.3 g, 199.0 mmols) in  
18 dichloromethane (55 ml) was added acetylchloride (15 g, 192 mmols) and  
19 1,2,3,4-tetrahydro-1,1-dimethylnaphthalene (24.4g, 152 mmols) in  
20 dichloromethane (20 ml) over 20 minutes. The reaction mixture was warmed  
21 to ambient temperature and stirred for 4 hours. Ice (200 g) was added to the  
22 reaction flask and the mixture diluted with ether (400 ml). The layers were  
23 separated and the organic phase washed with 10% HCl (50 ml), water (50  
24 ml), 10% aqueous sodium bicarbonate, and saturated aqueous NaCl (50 ml)  
25 before being dried over MgSO<sub>4</sub>. This solvent was removed by distillation to  
26 afford a yellow oil which was dissolved in benzene (50 ml).

27 To a cold (0°C) solution of acetic acid (240 ml) and acetic anhydride  
28 (120 ml) was added chromiumtrioxide (50 g, 503 mmols) in small portions  
29 over 20 minutes under argon. The mixture was stirred for 30 mins at 0°C and



1 diluted with benzene (120 ml). The benzene solution prepared above was  
2 added with stirring via an addition funnel over 20 minutes. After 8 hours, the  
3 reaction was quenched by careful addition of isopropanol (50 ml) at 0°C,  
4 followed by water (100 ml). After 15 minutes, the reaction mixture was  
5 diluted with ether (1100 ml) and water (200 ml), and then neutralized with  
6 solid sodium bicarbonate (200 g). The ether layer was washed with water (100  
7 ml), and saturated aqueous NaCl (2 x 100 ml), and dried over MgSO<sub>4</sub>.  
8 Removal of the solvent under reduced pressure afforded a mixture of the  
9 isomeric diketones which were separated by chromatography ( 5% EtOAc /  
10 hexanes). (Compound R): <sup>1</sup>H NMR (CDCl<sub>3</sub>) : δ 8.55 (1H, d, J = 2.0 Hz),  
11 8.13 (1H, dd, J = 2.0, 8.3 Hz), 7.53 (1H, d, J = 8.3 Hz), 2.77 (2H, t, J = 6.6  
12 Hz), 2.62 (3H, s), 2.05 (2H, t, J = 6.6 Hz), 1.41 (6H, s). (Compound S): <sup>1</sup>H  
13 NMR (CDCl<sub>3</sub>) : δ 8.10 (1H, d, J = 8.1 Hz), 8.02 (1H, d, J = 1.6 Hz), 7.82  
14 (1H, dd, J = 1.6, 8.1 Hz), 2.77 (2H, t, J = 7.1 Hz), 2.64 (3H, s), 2.05 (2H, t, J  
15 = 7.1 Hz), 1.44 (6H, s).

16 3,4-Dihydro-4,4-dimethyl-7-(2-(2-methyl-1,3-dioxolanyl))-1(2H)-naphthalenone  
17 (Compound T)

18 A mixture of 3,4-dihydro-4,4-dimethyl-7-acetyl- 1(2H)-naphthalenone  
19 (Compound R) (140.0 mg, 0.60 mmol), ethylene glycol (55.0 mg, 0.90 mmol),  
20 p- toluenesulfonic acid monohydrate (4 mg) and benzene (25 ml) was  
21 refluxed using a Dean-Stark apparatus for 12 hours. The reaction was  
22 quenched by the addition of 10% aqueous sodium bicarbonate, and extracted  
23 with ether (2 x 75 ml). The combined organic layers were washed with water  
24 (5 ml), and saturated aqueous NaCl (5 ml), and dried over MgSO<sub>4</sub>. Removal  
25 of the solvent under reduced pressure afforded the title compound as an oil.  
26 <sup>1</sup>H NMR (CDCl<sub>3</sub>) : δ 8.13 (1H, d, J = 2.0 Hz), 7.64 (1H, dd, J = 2.0, 8.2  
27 Hz), 7.40 (1H, d, J = 8.2 Hz), 3.97- 4.10 (2H, m), 3.70-3.83 (2H, m), 2.73  
28 (2H, t, J = 6.5 Hz), 2.01 (2H, t, J = 6.5 Hz), 1.64 (3H, s), 1.39 (6H, s).  
29 1,2,3,4-Tetrahydro-1-hydroxy-1-(4-methylphenyl)-4,4-dimethyl-7-(2-(2-

1 methyl-1,3-dioxolanyl)naphthalene (Compound U)

2 To a solution of 195.4 mg (1.00 mmol) p- tolulylmagnesiumbromide  
3 (1.0 ml; 1M solution in ether) in 2 ml THF was added a solution of 3,4-  
4 dihydro-4,4- dimethyl-7-(2-(2-methyl-1,3-dioxolanyl))-1(2H)- naphthalenone  
5 (Compound T) 135.0 mg, 0.52 mmol) in 5 ml THF. The solution was refluxed  
6 for 16 hours, cooled to room temperature, and diluted with ether (50 ml).  
7 The solution was washed with water (5 ml), saturated aqueous NH<sub>4</sub>Cl (5 ml),  
8 and dried over MgSO<sub>4</sub>. Removal of the solvents under reduced pressure and  
9 column chromatography (5% EtOAc / hexanes) afforded the title compound  
10 as a solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>) : δ 7.37 (2H, d), 7.21 (1H, s), 7.13 (2H, d, J  
11 = 8.5 Hz), 7.08 (2H, d, J = 8.5 Hz), 3.88- 3.99 (2H, m), 3.58-3.75 (2H, m),  
12 2.34 (3H, s), 2.12-2.30 (2H, m), 1.79-1.90 (1H, m), 1.57 (3H, s), 1.48-1.58  
13 (1H, m), 1.38 (3H, s), 1.31 (3H, s).

14 3,4-Dihydro-1-(4-methylphenyl)-4,4-dimethyl-7-acetylnaphthalene (Compound  
15 V)

16 A mixture of 1,2,3,4-tetrahydro-1-hydroxy-1-(4- methylphenyl)-4,4-  
17 dimethyl-7-(2-(2-methyl-1,3- dioxolanyl)naphthalene (Compound U) 130.0  
18 mg (0.38 mmol), p-toluenesulfonic acid monohydrate (4 mg) and benzene (5  
19 ml) was refluxed for 16 hours. Upon cooling to room temperature, the  
20 reaction mixture was diluted with ether (100 ml) and washed with 10%  
21 aqueous sodium bicarbonate, water, and saturated aqueous NaCl. The  
22 organic layer was dried over MgSO<sub>4</sub> and the solvents were removed under  
23 reduced pressure to give the title compound as a solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>) : δ  
24 7.83 (1H, dd, J = 1.8,8.0 Hz), 7.66 (1H, d, J = 1.8 Hz), 7.45 (1H, d, J = 8.0  
25 Hz), 7.25 (2H, d, J = 8.5 Hz), 7.22 (2H, d, J = 8.5 Hz), 6.03 (1H, t, J =  
26 6.3Hz), 2.47 (3H, s), 2.41 (3H, s), 2.37 (2H, d, J = 6.3Hz), 1.36 (6H, s).  
27 (E)-3-(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-naphthalenyl)-2-  
28 butenenitrile (Compound W)

29 To a slurry of NaH (48.0 mg, 2.00 mmol) in THF (6 ml), was added

1 diethylcyanomethylphosphonate (450.0 mg, 2.50 mmol). After 40 mins, a  
2 solution of 3,4-dihydro- 1-(4-methylphenyl)-4,4-dimethyl-7-acetylnaphthalene  
3 (Compound V) 95.0 mg, (0.33 mmol) in THF (4 ml) was added. The mixture  
4 was stirred for 16 hours, diluted with ether (100 ml), and washed with water,  
5 and saturated aqueous NaCl before being dried over MgSO<sub>4</sub>. Removal of  
6 the solvents under reduced pressure, and column chromatography (3%  
7 EtOAc / hexanes) afforded the title compound as a solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  
8  $\delta$  7.39 (1H, d, J = 1H), 7.32 (1H, dd, J = 2.0, 8.1Hz), 7.20-7.25 (4H, brs),  
9 7.15 (1H, d, J = 2.0 Hz), 6.03 (1H, t, J = 6.0 Hz), 5.44 (1H, s), 2.42 (3H, s),  
10 2.36 (2H, d, J = 6.0 Hz), 2.35 (3H, s), 1.35 (6H, s).

11 (E)-3-(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-naphthalenyl)-2-butenal  
12 (Compound X)

13 To a cold solution (-78°C) of (E)-3-(5,6-dihydro- 5,5-dimethyl-8-(4-  
14 methylphenyl)-2-naphthalenyl)-2- butenenitrile (Compound W) 84.0 mg, 0.29  
15 mmol) in dichloromethane (4 ml) was added 0.50 ml (0.50 mmol) of  
16 diisobutylaluminumhydride (1M solution in dichloromethane). After stirring  
17 for 1 hour, the reaction was quenched at -78°C by adding 2-propanol (1 ml)  
18 diluted with ether (100 ml). Upon warming to room temperature, the solution  
19 was washed with water, 10% HCl, and saturated aqueous NaCl. The organic  
20 layer was dried over MgSO<sub>4</sub> and the solvent removed under reduced pressure  
21 to give the title compound as an oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>) :  $\delta$  10.12 (1H, d, J  
22 = 7.9 Hz), 7.43 (2H, s), 7.19-7.28 (5H, m), 6.27 (1H, d, J = 7.9 Hz), 6.03  
23 (1H, t, J = 4.8 Hz), 2.47 (3H, s), 2.42 (3H, s), 2.37 (2H, d, J = 4.8 Hz), 1.37  
24 (6H, s).

25 Ethyl (E,E,E)-3-methyl-7-(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-  
26 naphthalenyl)-2,4,6-octatrienoate (Compound 51)

27 To a cold (-78°C) solution of diethyl-(E)-3- ethoxycarbonyl-2-  
28 methylallylphosphonate [prepared in accordance with *J. Org. Chem.* 39: 821  
29 (1974)] 264.0 mg, (1.00 mmol) in THF (2 ml) was added 26.0 mg (0.41 mmol,

1 0.65 ml) of n-butyllithium in hexanes (1.6 M solution) followed immediately  
2 by the addition of (E)-3-(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-  
3 naphthalen-yl)-2-butenal (Compound X) 82.0 mg, 0.26 mmol) in THF (3  
4 ml). After 1 hour, the reaction mixture was diluted with ether (60 ml),  
5 washed with water (5 ml), saturated aqueous NaCl (5 ml) and dried over  
6 MgSO<sub>4</sub>. After removal of the solvents under reduced pressure, the title  
7 compound was isolated as an oil by column chromatography (5% EtOAc /  
8 hexanes, followed by HPLC using 1% EtOAc / hexanes). <sup>1</sup>H NMR (acetone-  
9 d<sub>6</sub>) : δ 7.36-7.43 (2H, m), 7.18-7.27 (4H, m), 7.17 (1H, d, J = 1.7 Hz), 7.08  
10 (1H, dd, J = 11.2, 15.2 Hz), 6.46 (1H, d, J = 11.2 Hz), 6.38 (1H, d, J = 15.2  
11 Hz), 5.98 (1H, t, J = 4.7 Hz), 5.78 (1H, s), 4.10 (2H, q, J = 7.1 Hz), 2.35  
12 (3H, s), 2.33 (3H, s), 2.32 (2H, d, J = 4.7 Hz), 2.12 (3H, s), 1.31 (6H, s), 1.22  
13 (3H, t, J = 7.1 Hz).

14 (E,E,E)-3-methyl-7-(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-  
15 naphthalenyl)-2,4,6-octatrienoic acid (Compound 52)

16 To a solution of ethyl (E,E,E)-3-methyl-7-(5,6-dihydro-5,5-dimethyl-8-  
17 (4-methylphenyl)-2-naphthalenyl)-2,4,6-octatrienoate (Compound 51) 85.0  
18 mg, 0.20 mmol) in THF (1ml) and methanol (1ml) was added 12.0 mg (0.50  
19 mmol) of LiOH (0.5 ml, 1M solution). The mixture was stirred for 6 hours,  
20 diluted with ether (60 ml), acidified with 10% HCl (1 ml). The solution was  
21 washed with water, and saturated aqueous NaCl, before being dried over  
22 MgSO<sub>4</sub>. Removal of the solvents under reduced pressure afforded the title  
23 compound as a solid, which was purified by recrystallization from acetone.  
24 <sup>1</sup>H NMR (acetone-d<sub>6</sub>) : δ 7.35-7.45 (2H, m), 7.19-7.28 (4H, m), 7.17 (1H, d,  
25 J = 1.8 Hz), 7.09 (1H, dd, J = 11.5, 15.1 Hz), 6.48 (1H, d, J = 11.5 Hz), 6.42  
26 (1H, d, J = 15.1 Hz), 5.99 (1H, t, J = 4.7 Hz), 5.82 (1H, s), 2.36 (3H, s), 2.33  
27 (2H, d, J = 4.7 Hz), 2.32 (3H, s), 2.13 (3H, s), 1.32 (6H, s).

28 3,4-dihydro-4,4-dimethyl-7-nitro-1(2H)-naphthalenone (Compound Y)

29 To 1.7 ml (3.0g, 30.6 mmol, 18M) H<sub>2</sub>SO<sub>4</sub> at -5°C (ice-NaCl bath) was

1 slowly added 783.0 mg (4.49 mmol) of 3,4-dihydro-4,4-dimethyl-1(2H)-  
2 naphthalenone. A solution of 426.7 mg (6.88 mmol, 0.43 ml, 16M)  $\text{HNO}_3$ ,  
3 and 1.31g (0.013 mol, 0.74 ml, 18 M)  $\text{H}_2\text{SO}_4$  was slowly added. After 20  
4 minutes, ice was added and the resulting mixture extracted with EtOAc. The  
5 combined extracts were concentrated under reduced pressure to give a  
6 residue from which the title compound, a pale yellow solid, was isolated by  
7 column chromatography (10% EtOAc / hexanes).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) :  $\delta$   
8 8.83 (1H, d, J = 2.6 Hz), 8.31 (1H, dd, J = 2.8, 8.9 Hz), 7.62 (1H, d, J = 8.7  
9 Hz), 2.81 (2H, t, J = 6.5 Hz), 2.08 (2H, t, J = 6.5 Hz), 1.45 (6H, s).

10 3,4-dihydro-4,4-dimethyl-7-amino-1(2H)-naphthalenone (Compound Z)

11 A solution of 230.0 mg (1.05 mmol) 3,4-dihydro-4,4-dimethyl-7-  
12 nitro-1(2H)-naphthalenone (Compound Y) in 5.0 ml of EtOAc was stirred at  
13 room temperature with a catalytic amount of 10% Pd-C under 1 atm of  $\text{H}_2$   
14 for 24 hours. The catalyst was removed by filtration through a pad of Celite,  
15 and the filtrate concentrated under reduced pressure to give the title  
16 compound as a dark green oil.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) :  $\delta$  7.30 (1H, d, J = 2.7  
17 Hz), 7.22 (1H, d, J = 8.4 Hz), 6.88 (1H, dd, J = 2.7, 8.5 Hz), 2.70 (2H, t, J =  
18 6.6 Hz), 1.97 (2H, t, J = 6.6 Hz), 1.34 (6H, s).

19 Ethyl 4-[(5,6,7,8-tetrahydro-5,5-dimethyl-8-oxo-2-naphthalenyl)azo]-benzoate  
20 (Compound AA)

21 To a solution of 198.7 mg (1.05 mmol) 3,4-dihydro-4,4-dimethyl-7-  
22 amino-1(2H)-naphthalenone (Compound Z) in 5.0 ml glacial acetic acid was  
23 added 180.0 mg (1.00 mmol) of ethyl 4-nitrosobenzoate. The resulting  
24 solution was stirred overnight at room temperature, and then concentrated  
25 under reduced pressure. The product was isolated from the residual oil as a  
26 red solid, by column chromatography (15% EtOAc - hexanes).  $^1\text{H}$  NMR  
27 ( $\text{CDCl}_3$ ) :  $\delta$  8.57 (1H, d, J = 2.0 Hz), 8.19 (2H, d, J = 8.4 Hz), 8.07 (1H, d,  
28 J = 8.0 Hz), 7.94 (2H, d, J = 8.4 Hz), 7.58 (1H, d, J = 8.6 Hz), 4.41 (2H, q,  
29 J = 7.1 Hz), 2.79 (2H, t, J = 6.6 Hz), 2.07 (2H, t, J = 7.02 Hz), 1.44 (6H, s),

1 1.42 (3H, t, J = 7.1 Hz).

2 Ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(trifluoromethylsulfonyl)oxy-2-  
3 naphthalenyl)azo]-benzoate (Compound BB)

4 To a solution of 90.4 mg sodium bis(trimethylsilyl)amide (0.48 mmol,  
5 0.48 ml of a 1.0 M THF solution) in 2.0 ml THF at -78°C, was added 153.0  
6 mg (0.437 mmol) of ethyl 4-[(5,6,7,8-tetrahydro-5,5-dimethyl-8-oxo-2-  
7 naphthalenyl)azo]-benzoate (Compound AA) in 2.0 ml THF. The dark red  
8 solution was stirred at -78°C for 30 minutes and then 204.0 mg (0.520 mmol)  
9 of 2-[N,N-bis(trifluoromethylsulfonyl)amino]-5-chloropyridine was added as a  
10 solution in 2.0 ml THF. The reaction mixture was allowed to warm to room  
11 temperature and after 3 hours it was quenched by the addition of H<sub>2</sub>O. The  
12 organic layer was concentrated to a red oil under reduced pressure. The  
13 product was isolated by column chromatography (25% EtOAc / hexanes) as a  
14 red oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>) : δ 8.21 (2H, d, J = 8.6 Hz), 7.96 (2H, d, J = 8.6  
15 Hz), 7.94 (2H, m), 7.49 (1H, d, J = 8.2 Hz), 6.08 (1H, t, J = 2.5 Hz), 4.42  
16 (2H, q, J = 7.1 Hz), 2.49 (2H, d, J = 4.8 Hz), 1.44 (3H, t, J = 7.1 Hz), 1.38  
17 (6H, s).

18 Ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-naphthalenyl)azo]-  
19 benzoate (Compound 46a)

20 A solution of 4-lithiotoluene was prepared by the addition of 62.9 mg  
21 (0.58 ml, 0.98 mmol) of t-butyl lithium (1.7 M solution in pentane) to a cold  
22 solution (-78°C) of 84.0 mg (0.491 mmol) of 4-bromotoluene in 1.0 ml of  
23 THF. After stirring for 30 minutes a solution of 107.0 mg (0.785 mmol) of  
24 zinc chloride in 2.0 ml of THF was added. The resulting solution was  
25 warmed to room temperature, stirred for 30 minutes, and added via cannula  
26 to a solution of 94.7 mg (0.196 mmol) of ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-  
27 (trifluoromethylsulfonyl)oxy-2-naphthalenyl)azo]-benzoate (Compound BB)  
28 and 25 mg (0.02 mmol) of tetrakis(triphenylphosphine)palladium(0) in 2.0 ml  
29 of THF. The resulting solution was heated at 50°C for 1.5 hours, cooled to

1 room temperature and diluted with sat. aqueous  $\text{NH}_4\text{Cl}$ . The mixture was  
2 extracted with EtOAc (40 ml) and the combined organic layers were washed  
3 with water and brine. The organic phase was dried over  $\text{Na}_2\text{SO}_4$ ,  
4 concentrated in vacuo, and the title compound isolated as a red solid by  
5 column chromatography (25% EtOAc-hexanes)  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) :  $\delta$  8.21  
6 (2H, d,  $J = 8.6$  Hz), 7.96 (2H, d,  $J = 8.6$  Hz), 7.94 (2H, m), 7.49 (1H, d,  $J =$   
7 8.2 Hz), 6.08 (1H, t,  $J = 2.5$  Hz), 4.42 (2H, q,  $J = 7.1$  Hz), 2.49 (2H, d,  $J =$   
8 4.8 Hz), 1.44 (3H, t,  $J = 7.1$  Hz), 1.38 (6H, s).

9 4-[(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-naphthalenyl)azo]-benzoic  
10 acid (Compound 46b)

11 To a solution of ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(4-  
12 methylphenyl)-2-naphthalenyl)azo]-benzoate (Compound 46a) 16.5 mg, 0.042  
13 mmol) in THF (2 ml) and ethanol (1ml) was added 80.0 mg (2.00 mmol) of  
14 NaOH (2.0 ml, 1M aqueous solution). The mixture was stirred for 12 hours  
15 at room temperature, acidified with 10% HCl, and extracted with EtOAc.  
16 The combined organic layers were washed with water, and saturated aqueous  
17 NaCl, then dried over  $\text{MgSO}_4$ . Removal of the solvents under reduced  
18 pressure, and recrystallization of the residue from EtOAc / hexane, afforded  
19 the title compound as a red solid.  $^1\text{H}$  NMR (acetone- $d_6$ ) :  $\delta$  8.19 (2H, d,  $J$   
20 = 8.4 Hz), 7.92 (2H, d,  $J = 8.5$  Hz), 7.88 (2H, dd,  $J = 2.1, 6.1$  Hz), 7.66 (1H,  
21 s), 7.64 (2H, d,  $J = 2.3$  Hz), 7.28 (4H, d,  $J = 3.0$  Hz), 6.09 (1H, t,  $J = 2.5$   
22 Hz), 2.42 (2H, d,  $J = 4.8$  Hz), 2.39 (3H, s), 1.40 (6H, s).

23 6-(2-Trimethylsilyl)ethynyl-2,3-dihydro-3,3-dimethyl-1H-inden-1-one  
24 (Compound CC)

25 To a solution of 815.0 mg (3.41 mmol) 6-bromo-2,3-dihydro-3,3-  
26 dimethyl-1H-inden-1-one (See Smith et al. Org. Prep. Proced. Int. 1978 10  
27 123-131) in 100 ml of degassed  $\text{Et}_3\text{N}$  (sparged with argon for 20 min) was  
28 added 259.6 mg (1.363 mmol) of copper(I) iodide, 956.9 mg (1.363 mmol) of  
29 bis(triphenylphosphine)palladium(II)chloride, and 3.14 g (34.08 mmol) of

1 (trimethylsilyl)acetylene. This mixture was heated at 70°C for 42 hours,  
2 cooled to room temperature, and filtered through a pad of silica gel and  
3 washed with ether. The filtrate was washed with water, 1 M HCl, water, and  
4 finally with saturated aqueous NaCl before being dried over  $\text{MgSO}_4$ .  
5 Concentration of the solution under reduced pressure, followed by column  
6 chromatography (silica gel; 10%  $\text{Et}_2\text{O}$  - hexanes) afforded the title compound  
7 as a brown oil.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.79(1H, d,  $J$  = 1.4 Hz),  
8 7.69 (1H, dd,  $J$  = 1.6, 8.3 Hz), 7.42 (1H, d,  $J$  = 8.5 Hz), 2.60 (2H, s), 1.41  
9 (6H, s), 0.26 (9H, s).

10 6-Ethynyl-2,3-dihydro-3,3-dimethyl-1H-inden-1-one (Compound DD)

11 To a solution of 875.0 mg (3.41 mmol) 6-(2- trimethylsilyl)ethynyl-2,3-  
12 dihydro-3,3-dimethyl-1H-inden-1-one (Compound CC) in 28 ml of MeOH,  
13 was added 197.3 mg (1.43 mmol) of  $\text{K}_2\text{CO}_3$  in one portion. After stirring for  
14 6 hours at room temperature the mixture was filtered though a pad of Celite  
15 and the filtrate concentrated under reduced pressure. The residual oil was  
16 placed on a silica gel column and eluted with 5% EtOAc-hexanes to give the  
17 title product as a colorless oil.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.82 (1H, s),  
18 7.72 (1H, dd,  $J$  = 1.6, 7.8 Hz), 7.47 (1H, d,  $J$  = 8.4 Hz), 3.11 (1H, s), 2.61  
19 (2H, s), 1.43 (6H, s).

20 Ethyl 4-[2-(5,6-dihydro-5,5-dimethyl-7-oxo-2-indenyl)ethynyl]benzoate  
21 (Compound EE)

22 A solution of 280.0 mg (1.520 mmol) 6-ethynyl-2,3- dihydro-3,3-  
23 dimethyl-1H-inden-1-one (Compound DD) and 419.6 mg (1.520 mmol) ethyl  
24 4-iodobenzoate in 5 ml  $\text{Et}_3\text{N}$  was sparged with argon for 40 minutes. To this  
25 solution was added 271.0 mg (1.033 mmol) of triphenylphosphine, 53.5 mg  
26 (0.281 mmol) of copper(I) iodide, and 53.5 mg (0.076 mmol) of  
27 bis(triphenylphosphine)palladium(II)-chloride. The resulting mixture was  
28 heated to reflux for 2.5 hours, cooled to room temperature, and diluted with  
29  $\text{Et}_2\text{O}$ . After filtration through a pad of Celite, the filtrate was washed with



1 H<sub>2</sub>O, 1 M HCl, H<sub>2</sub>O, and saturated aqueous NaCl, then dried over MgSO<sub>4</sub>,  
2 and concentrated under reduced pressure. The title compound was isolated as  
3 a pale-yellow solid by column chromatography (15% EtOAc-hexanes). <sup>1</sup>H  
4 NMR (300 MHz, d<sub>6</sub>-acetone): δ 8.05 (2H, d, J = 8.6 Hz), 7.87 (1H, dd, J =  
5 1.4, 8.1 Hz), 7.75 (2H, m), 7.70 (2H, d, J = 8.5 Hz), 4.36 (2H, q, J = 7.1 Hz),  
6 2.60 (2H, s), 1.45 (6H, s), 1.37 (3H, t, J = 7.1 Hz).

7 Ethyl 4-[2-(1,1-dimethyl-3-(trifluoromethyl-sulfonyl)oxy-5-  
8 indenyl)ethynyl]benzoate (Compound FF)

9 A solution of 88.0 mg (0.48 mmol) of sodium bis(trimethylsilyl)amide  
10 in 0.5 ml THF was cooled to -78°C and 145.0 mg (0.436 mmol) of ethyl 4-[2-  
11 (5,6-dihydro-5,5-dimethyl-7-oxo-2-indenyl)ethynyl]benzoate (Compound EE)  
12 was added as a solution in 1.0 ml THF. After 30 minutes 181.7 mg (0.480  
13 mmol) of 2-(N,N-bis(trifluoromethylsulfonyl)amino)-5-chloro-pyridine was  
14 added as a solution in 1.0 ml THF. The reaction was allowed to slowly warm  
15 to room temperature and quenched after 5 hours by the addition of saturated  
16 aqueous NH<sub>4</sub>Cl. The mixture was extracted with EtOAc, and the combined  
17 organic layers washed with 5% aqueous NaOH, H<sub>2</sub>O, and saturated aqueous  
18 NaCl, then dried (MgSO<sub>4</sub>) and concentrated under reduced pressure. The  
19 product was isolated as a colorless solid by column chromatography (10%  
20 Et<sub>2</sub>O-hexanes). <sup>1</sup>H NMR (300 MHz, d<sub>6</sub>-acetone): δ 8.05 (2H, d, J = 8.3  
21 Hz), 7.69 (2H, d, J = 8.4 Hz), 7.63 (2H, s), 7.55 (1H, s), 4.36 (2H, q, J = 7.1  
22 Hz), 1.44 (6H, s), 1.37 (3H, t, J = 7.1 Hz).

23 4-[(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-  
24 naphthalenyl)ethynyl]benzoic acid (Compound 60)

25 A solution of 142.6 mg (0.339 mmol) of ethyl 4-[(5,6-dihydro-5,5-  
26 dimethyl-8-(4-methylphenyl)-2-naphthalenyl)ethynyl]benzoate (Compound 1)  
27 and 35.6 mg (0.848 mmol) of LiOH·H<sub>2</sub>O in 12 ml of THF/water (4:1, v/v),  
28 was stirred overnight at room temperature. The reaction mixture was  
29 extracted with hexanes, and the hexane fraction extracted with 5% aqueous

1 NaOH. The aqueous layers were combined and acidified with 1M HCl, and  
2 then extracted with EtOAc and Et<sub>2</sub>O. The combined organic layers were  
3 dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo to give the title compound as a  
4 colorless solid. <sup>1</sup>H NMR (d<sub>6</sub>-DMSO): δ 7.91 (2H, d, J = 8.4 Hz), 7.60 (2H,  
5 d, J = 8.4 Hz), 7.47 (2H, s), 7.23 (4H, q, J = 8.1 Hz), 7.01 (1H, s), 6.01 (1H,  
6 t, J = 4.6 Hz), 2.35 (3H, s), 2.33 (2H, d, J = 4.8 Hz), 1.30 (6H, s).

7 4-[(5,6-dihydro-5,5-dimethyl-8-phenyl-2-naphthalenyl)ethynyl]benzoic acid  
8 (Compound 60a)

9 Employing the same general procedure as for the preparation of 4-  
10 [(5,6-dihydro-5,5-dimethyl-8-(2-thiazolyl)-2-naphthalenyl)ethynyl]benzoic acid  
11 (Compound 30a), 27.0 mg (0.07 mmol) of ethyl 4-[(5,6-dihydro-5,5-  
12 dimethyl-8-phenyl-2-naphthalenyl)ethynyl]benzoate (Compound 1a) was  
13 converted into the title compound (colorless solid) using 5.9 mg (0.14 mmol)  
14 of LiOH in H<sub>2</sub>O. PMR (d<sub>6</sub>-DMSO): δ 1.31 (6H, s), 2.35 (2H, d, J = 4.5 Hz),  
15 6.05 (1H, t, J = J = J = 4.5 Hz), 7.00 (1H, s), 7.33 (2H, d, J = 6.2 Hz), 7.44  
16 (4H, m), 7.59 (2H, d, J = 8.1 Hz), 7.90 (2H, d, J = 8.1 Hz).

17 4-[(5,6-Dihydro-5,5-dimethyl-8-(4-(1,1-dimethylethyl)phenyl)-2-  
18 naphthalenyl)ethynyl]benzoic acid (Compound 61)

19 A solution of 80.0 mg (0.173 mmol) of ethyl 4-[(5,6-dihydro-5,5-  
20 dimethyl-8-(4-(1,1-dimethylethyl)phenyl)-2-naphthalenyl)ethynyl]benzoate  
21 (Compound 6) and 18.1 mg (0.432 mmol) of LiOH-H<sub>2</sub>O in 6 ml of  
22 THF/water (3:1, v/v), was stirred overnight at room temperature. The  
23 reaction mixture was extracted with hexanes, and the remaining aqueous layer  
24 acidified with 1M HCl, and then extracted with EtOAc. The combined  
25 organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo to give the  
26 title compound as a colorless solid. <sup>1</sup>H NMR (d<sub>6</sub>-DMSO): δ 7.82 (2H, d, J  
27 = 8.2 Hz), 7.44 (6H, m), 7.25 (2H, d, J = 8.3 Hz), 7.02 (1H, s), 6.01 (1H, t, J  
28 = 4.6 Hz), 2.32 (2H, d, J = 4.7 Hz), 1.32 (9H, s), 1.29 (6H, s).

29 Ethyl 2-fluoro-4-[(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-

1 naphthalenyl)thiocarbonyl]amino]-benzoate (Compound 62)

2 A solution of 54.4 mg (0.119 mmol) ethyl 2-fluoro-4-[[[(5,6-dihydro-5,5-  
3 dimethyl-8-(4-methylphenyl)-2-naphthalenyl)carbonyl]amino]-benzoate  
4 (Compound 40) and 57.7 mg (0.143 mmol) of [2,4-bis(4-methoxyphenyl)-1,3-  
5 dithia-2,4-diphosphetane-2,4-disulfide] (Lawesson's Reagent) in 12.0 ml of  
6 benzene was refluxed overnight. Upon cooling to room temperature, the  
7 mixture was filtered and the filtrate concentrated under reduced pressure.  
8 The title compound was isolated by column chromatography (10 to 25%  
9 EtOAc / hexanes) as a yellow solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 9.08 (1H, s), 7.92  
10 (1H, br s), 7.90 (1H, t, J = 8.2 Hz), 7.66 (1H, dd, J = 2.0, 6.0 Hz), 7.38 (3H,  
11 m), 7.18 (4H, m), 6.01 (1H, t, J = 4.7 Hz), 4.35 (2H, q, J = 7.1 Hz), 2.36  
12 (3H, s), 2.33 (2H, d, J = 4.7 Hz), 1.38 (3H, t, J = 7.1 Hz), 1.33 (6H, s).

13 2-fluoro-4-[[[(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-  
14 naphthalenyl)thiocarbonyl]amino]-benzoic acid (Compound 63)

15 To a solution of 46.5 mg (0.098 mmol) ethyl 2-fluoro-4-[[[(5,6-  
16 dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-naphthalenyl)thiocarbonyl]amino]-  
17 benzoate (Compound 62) in 1.0 ml EtOH and 1.0 ml of THF was added 55  
18 mg NaOH (1.4 mmol) and 1.0 ml of H<sub>2</sub>O. After stirring at room temperature  
19 for overnight EtOAc was added, and the reaction quenched by the addition  
20 of 10% HCl. Extraction with EtOAc was followed by washing of the  
21 combined organic layers with H<sub>2</sub>O, saturated aqueous NaCl, and drying over  
22 MgSO<sub>4</sub>. Removal of the solvent under reduced pressure provided a solid  
23 which after crystallization from CH<sub>3</sub>CN afforded the title compound as a  
24 pale-yellow solid. <sup>1</sup>H NMR (d<sub>6</sub>-acetone): δ 11.05 (1H, s), 8.02 (1H, m), 7.99  
25 (1H, t, J = 8.3 Hz), 7.75 (1H, m), 7.69 (1H, dd, J = 2.0, 6.1 Hz), 7.52 (1H,  
26 s), 7.46 (1H, d, J = 8.1 Hz), 7.21 (4H, m), 6.04 (1H, t, J = 4.8 Hz), 2.37 (2H,  
27 d, J = 4.8 Hz), 2.33 (3H, s), 1.36 (6H, s).

28 Ethyl 5',6'-dihydro-5',5'-dimethyl-8'-(4-methylphenyl)-[2,2'-binaphthalene]-6-  
29 carboxylate (Compound 64)

1 A solution of 3,4-dihydro-1-(4-methylphenyl)-4,4- dimethyl-7-  
2 bromonaphthalene Compound D) 0.45 g, 1.40 mmol) and THF (2.1 ml) was  
3 added to magnesium turnings (0.044 g, 1.82 mmol) at room temperature  
4 under argon. Two drops of ethylene dibromide were added, and the solution,  
5 which slowly became cloudy and yellow, was heated to reflux for 1.5 hours.  
6 In a second flask was added zinc chloride (0.210 g, 1.54 mmol), which was  
7 melted under high vacuum, cooled to room temperature and dissolved in  
8 THF (3 ml). The Grignard reagent was added to the second flask and, after  
9 30 minutes at room temperature, a solution of ethyl 6-bromo-2-  
10 naphthalinecarboxylate (Compound N) 0.293 g, (1.05 mmol) and THF (2 ml)  
11 were added. In a third flask was prepared a solution of  $\text{Ni}(\text{PPh}_3)_4$  and THF  
12 as follows: To a solution of  $\text{NiCl}_2(\text{PPh}_3)_2$  (0.82 g, 1.25 mmol) and  $\text{PPh}_3$  (0.66  
13 g, 2.5 mmol) in THF (3.5 ml) was added a 1M solution of  
14 diisobutylaluminum hydride and hexanes (2.5 ml, 2.5 mmol), and the resulting  
15 solution diluted with THF to a total volume of 15 ml and stirred at room  
16 temperature for 15 minutes. Three 0.60 ml aliquots of the  $\text{Ni}(\text{PPh}_3)_4$  solution  
17 were added at 15 minutes intervals to the second flask. The resulting  
18 suspension was stirred at room temperature for 2 hours. The reaction was  
19 quenched by the addition of 5 ml 1N aqueous HCl and stirred for 1 hour  
20 before extracting the products with ethyl acetate. The organic layers were  
21 combined, washed with brine, dried ( $\text{MgSO}_4$ ), filtered and the solvent  
22 removed in-vacuo. The residue was crystalized from hexanes to give 130 mg  
23 of pure material. The mother liquor was concentrated under reduced  
24 pressure and the residue purified by silica gel chromatography (95:5-  
25 hexanes:ethyl acetate) to give an additional 170 mg of the title compound  
26 (overall yield = 300 mg, 64 %) as a colorless solid.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.57  
27 (s, 1H), 8.05 (dd, 1H,  $J = -1.7, 8.0$  Hz), 7.84-7.95 (overlapping d's, 3H), 7.66  
28 (dd, 1H,  $J = 1.7, 8.5$  Hz), 7.58 (dd, 1H,  $J = 2.0, 8.0$  Hz), 7.48 (d, 1H,  $J = 8.0$   
29 Hz), 7.43 (d, 1H,  $J = 2.0$  Hz), 7.32 (d, 2H,  $J = 8.0$  Hz), 7.21 (d, 2H,  $J = 8.0$

1 Hz), 6.04 (t, 1H, J = 4.8 Hz), 4.44 (q, 2H, J = 7.1 Hz), 2.40 (s, 3H), 2.39 (d,  
2 2H, J = 4.8 Hz), 1.45 (t, 3H, J = 7.1 Hz), 1.39 (s, 6H).

3 5',6'-Dihydro-5',5'-dimethyl-8'-(4-methylphenyl)-[2,2'-binaphthalene]-6-  
4 carboxylic acid (Compound 65)

5 A solution of ethyl 5',6'-dihydro-5',5'-dimethyl- 8'-(4-methylphenyl)-  
6 [2,2'-binaphthalene]-6-carboxylate (Compound 64) 0.19 g, 0.43 mmol), EtOH  
7 (8 ml) and 1N aqueous NaOH (2 ml) was heated to 60°C for 3 hours. The  
8 solution was cooled to 0°C and acidified with 1N aqueous HCl. The product  
9 was extracted into ethyl acetate, and the organic layers combined, washed  
10 with water, brine, dried (MgSO<sub>4</sub>), filtered and the solvent removed in-vacuo.  
11 The residue was recrystallized from THF/ethyl acetate at 0°C to give 35 mg of  
12 pure material. The mother liquor was concentrated under reduced pressure  
13 and the residue purified by silica gel chromatography (100% ethyl acetate) to  
14 give an additional 125 mg of the title compound (overall yield = 160 mg, 90  
15 %) as a colorless solid. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ 8.57 (s, 1H), 8.11 (d, 1H, J  
16 = 8.7 Hz), 7.96-7.82 (overlapping d's, 3H), 7.65 (d, 2H, J = 7.6 Hz), 7.50 (d,  
17 1H, J = 7.9 Hz), 7.28 (s, 1H), 7.26 (d, 2H, J = 8.3 Hz), 7.21 (d, 2H, J = 8.3  
18 Hz), 6.01 (t, 1H, J = 4.5 Hz), 3.34 (br s, 1H), 2.31 (s, 3H), 2.31 (d, 2H, J =  
19 4.5 Hz), 1.31 (s, 6H).

20 Ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(2-furyl)-2-naphthalenyl)ethynyl]benzoate  
21 (Compound 66)

22 Employing the same general procedure as for the preparation of ethyl  
23 4-[(5,6-dihydro-5,5-dimethyl-8-(4-methylphenyl)-2-  
24 naphthalenyl)ethynyl]benzoate (Compound 1), 250.0 mg (0.52 mmol) of ethyl  
25 4-[(5,6-dihydro-5,5-dimethyl-8-(trifluoromethylsulfonyl)oxy-2-  
26 naphthalenyl)ethynyl]benzoate (Compound G) was converted into the title  
27 compound (colorless solid) using 142.4 mg (1.045 mmol) of zinc chloride,  
28 24.1 mg (0.02 mmol) of tetrakis(triphenylphosphine)palladium(0) and 2-  
29 lithiofuran (prepared by the addition of 53.4 mg (0.52 ml, 0.78 mmol) of n-

1 butyllithium (1.5M solution in hexane) to a cold solution (-78°C) of 53.4 mg  
2 (0.784 mmol) of furan in 1.0 ml of THF). PMR (CDCl<sub>3</sub>): δ 1.32 (6H, s), 1.41  
3 (3H, t, J = 7.1 Hz), 2.35 (2H, d, J = 5.0 Hz), 4.39 (2H, q, J = 7.1 Hz), 6.41  
4 (1H, t, J = 5.0 Hz), 6.50 (2H, s), 7.36 (1H, d, J = 8.0 Hz), 7.45 (1H, dd, J =  
5 1.7, 8.0 Hz), 7.49 (1H, s), 7.57 (2H, d, J = 8.2 Hz), 7.63 (1H, d, J = 1.7 Hz),  
6 8.02 (2H, d, J = 8.2 Hz).

7 4-[(5,6-dihydro-5,5-dimethyl-8-(2-furyl)-2-naphthalenyl)ethynyl]benzoic acid  
8 (Compound 67)

9 Employing the same general procedure as for the preparation of 4-  
10 [(5,6-dihydro-5,5-dimethyl-8-(2-thiazolyl)-2-naphthalenyl)ethynyl]benzoic acid  
11 (Compound 30a), ethyl 4-[(5,6-dihydro-5,5-dimethyl-8-(2-furyl)-2-  
12 naphthalenyl)ethynyl]benzoate (Compound 66) was converted into the title  
13 compound (colorless solid) using 16.0 mg (0.38 mmol) of LiOH in H<sub>2</sub>O.  
14 PMR (d<sub>6</sub>-DMSO): δ 1.26 (6H, s), 2.33 (2H, d, J = 4.9 Hz), 6.41 (1H, t, J =  
15 4.9 Hz), 6.60 (2H, m), 7.45-7.53 (3H, m), 7.64 (2H, d, J = 8.3 Hz), 7.75 (1H,  
16 d, J = 1.6 Hz), 7.93 (2H, d, J = 8.3 Hz).

17 3,4-dihydro-4,4-dimethyl-7-acetyl-1(2H)-naphthalenone (Compound 100C)  
18 and 3,4-dihydro-4,4-dimethyl-6-acetyl-1(2H)-naphthalenone (Compound  
19 100D)

20 To a cold (0° C) mixture of aluminum chloride (26.3 g, 199.0 mmols)  
21 in dichloromethane (55 ml) was added acetylchloride (15 g, 192 mmols) and  
22 1,2,3,4-tetrahydro-1,1-dimethylnaphthalene (24.4g, 152mmols) in  
23 dichloromethane (20 ml) over 20 minutes. The reaction mixture was warmed  
24 to ambient temperature and stirred for 4 hours. Ice (200 g) was added to the  
25 reaction flask and the mixture diluted with ether (400 ml). The aqueous and  
26 organic layers were separated and the organic phase was washed with 10%  
27 HCl (50 ml), water (50 ml), 10% aqueous sodium bicarbonate, and saturated  
28 aqueous NaCl (50 ml) and then dried over MgSO<sub>4</sub>. The solvent was removed  
29 by distillation to afford a yellow oil which was dissolved in benzene (50 ml).

1 To a cold (0° C) solution of acetic acid (240 ml) and acetic anhydride  
2 (120 ml) was added chromium trioxide (50 g, 503 mmols) in small portions  
3 over 20 minutes under argon. The mixture was stirred for 30 minutes at 0° C  
4 and diluted with benzene (120 ml). The benzene solution prepared above was  
5 added with stirring via an addition funnel over 20 minutes. After 8 hours, the  
6 reaction was quenched by the careful addition of isopropanol (50 ml) at 0° C,  
7 followed by water (100 ml). After 15 minutes, the reaction mixture was  
8 diluted with ether (1100 ml) and water (200 ml), and then neutralized with  
9 solid sodium bicarbonate (200 g). The ether layer was washed with water  
10 (100 ml), and saturated aqueous NaCl (2 x 100 ml), and dried over MgSO<sub>4</sub>.  
11 Removal of the solvent under reduced pressure afforded a mixture of the  
12 isomeric diketones which were separated by chromatography ( 5% EtOAc /  
13 hexanes). (Compound 100C): <sup>1</sup>H NMR (CDCl<sub>3</sub>) : δ 8.55 (1H, d, J = 2.0  
14 Hz), 8.13 (1H, dd, J = 2.0, 8.3 Hz), 7.53 (1H, d, J = 8.3 Hz), 2.77 (2H, t, J  
15 = 6.6 Hz), 2.62 (3H, s), 2.05 (2H, t, J = 6.6 Hz), 1.41 (6H, s). (Compound  
16 100D): <sup>1</sup>H NMR (CDCl<sub>3</sub>) : δ 8.10 (1H, d, J = 8.1 Hz), 8.02 (1H, d, J = 1.6  
17 Hz), 7.82 (1H, dd, J = 1.6; 8.1 Hz), 2.77 (2H, t, J = 7.1 Hz), 2.64 (3H, s),  
18 2.05 (2H, t, J = 7.1 Hz), 1.44 (6H, s).  
19 3,4-dihydro-4,4-dimethyl-6-(2-(2-methyl-1,3-dioxolanyl))-1(2H)-naphthalenone  
20 (Compound 100E)

21 A solution of 1.80 g (8.34 mmol) of a 1:5 mixture of 3,4-dihydro-4,4-  
22 dimethyl-7-acetyl-1(2H)- naphthalenone (Compound 100C); and 3,4-  
23 dihydro-4,4- dimethyl-6-acetyl-1(2H)-naphthalenone (Compound 100D) in 50  
24 ml benzene was combined with 517.7 mg (8.34 mmol) of ethylene glycol and  
25 20.0 mg (0.11 mmol) of p- toluenesulfonic acid monohydrate. The resulting  
26 solution was heated to reflux for 18 hours, cooled to room temperature, and  
27 concentrated under reduced pressure. The title compound was isolated by  
28 column chromatography (10% EtOAc - hexanes) as a colorless oil. <sup>1</sup>H NMR  
29 (CDCl<sub>3</sub>) : δ 8.01 (1H, d, J = 8.2 Hz), 7.51 (1H, s), 7.43 (1H, dd, J = 1.7, 6.4

1 Hz), 4.07 (2H, m), 3.79 (2H, m), 2.74 (2H, t, J = 6.5 Hz), 2.04 (2H, t, J = 7.1  
2 Hz), 1.67 (3H, s), 1.46 (6H, s).

3 1,2,3,4-tetrahydro-1-hydroxy-1-(4-methylphenyl)-4,4-dimethyl-6-(2-(2-  
4 methyl-1,3-dioxolanyl))naphthalene (Compound 100F)

5 To a solution of 496.2 mg (2.54 mmol) p-tolylmagnesiumbromide in  
6 20 ml THF (2.54 ml; 1M solution in ether) was added a solution of 3,4-  
7 dihydro-4,4-dimethyl-6-(2-(2-methyl-1,3-dioxolan-yl))-1(2H)-naphthalenone  
8 (Compound 100E, 200.0 mg, 0.769 mmol) in THF (5 ml). The solution was  
9 refluxed for 16 hours, cooled to room temperature, and washed with water,  
10 saturated aqueous NH<sub>4</sub>Cl, and dried over MgSO<sub>4</sub>. Removal of the solvents  
11 under reduced pressure and column chromatography (10% EtOAc / hexanes)  
12 afforded the title compound as a colorless solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.49  
13 (1H, d, J = 1.7 Hz), 7.19 (2H, m), 7.10 (2H, d, J = 7.9 Hz), 7.04 (1H, d, J =  
14 8.2 Hz), 4.05 (2H, m), 3.80 (2H, m), 2.34 (3H, s), 2.21 (1H, m), 2.10 (1H, m),  
15 1.88 (1H, m), 1.65 (3H, s), 1.54 (1H, m), 1.39 (3H, s), 1.33 (3H, s).

16 3,4-dihydro-1-(4-methylphenyl)-4,4-dimethyl-6-acetylnaphthalene (Compound  
17 100G)

18 A solution of 1,2,3,4-tetrahydro-1-hydroxy-1-(4-methylphenyl)-4,4-  
19 dimethyl-6-(2-(2-methyl-1,3-dioxolanyl))naphthalene (Compound 100F 160.0  
20 mg, 0.52 mmol), p-toluenesulfonic acid monohydrate (4 mg) and 30 ml  
21 benzene was refluxed for 12 hours. After cooling to room temperature, the  
22 reaction mixture was diluted with ether (100 ml) and washed with 10%  
23 aqueous sodium bicarbonate, water, and saturated aqueous NaCl. The  
24 organic layer was dried over MgSO<sub>4</sub> and the solvents were removed under  
25 reduced pressure to give the title compound, which was isolated by column  
26 chromatography (10% EtOAc-hexanes) as a yellow oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ  
27 7.97 (1H, d, J = 1.8 Hz), 7.67 (1H, dd, J = 1.7, 6.4 Hz), 7.22 (4H, s), 7.13  
28 (1H, d, J = 8.1 Hz), 6.10 (1H, t, J = 4.5 Hz), 2.59 (3H, s), 2.40 (3H, s), 2.38  
29 (2H, d, J = 4.7 Hz), 1.38 (6H, s).



1 4-[3-oxo-3-(7,8-dihydro-5-(4-methylphenyl)-8,8-dimethyl-2-naphthalenyl)-1-  
2 propenyl]-benzoic acid (Compound 101)

3 To a solution of 78.7 mg (0.272 mmol) 3,4-dihydro- 1-(4-  
4 methylphenyl)-4,4-dimethyl-6-acetylnaphthalene (Compound 100G) in 4.0 ml  
5 of MeOH was added 53.1 mg (0.354 mmol) of 4-carboxy benzaldehyde, and  
6 80. mg (2.00 mmol; 2.0 ml of 1M aqueous NaOH). The resulting solution  
7 was stirred at room temperature for 12 hours, concentrated under reduced  
8 pressure, and the residual oil dissolved in EtOAc. The solution was treated  
9 with 10% HCl, and the organic layer was washed with H<sub>2</sub>O, and saturated  
10 aqueous NaCl, then dried over Na<sub>2</sub>SO<sub>4</sub>. Removal of the solvents under  
11 reduced pressure gave the title compound as a colorless solid which was  
12 purified by recrystallization from CH<sub>3</sub>CN. <sup>1</sup>H NMR (acetone-d<sub>6</sub>) : δ 8.00  
13 (7H, m), 7.83 (1H, d, J = 15.6 Hz), 7.24 (4H, s), 7.13 (1H, d, J = 8.1 Hz),  
14 6.12 (1H, t, J = 4.5 Hz), 2.42 (2H, d, J = 4.8 Hz), 2.38 (3H, s), 1.41 (6H, s).

15 3,4-dihydro-1-phenyl-4,4-dimethyl-6-acetylnaphthalene (Compound 100H)

16 To a solution of 508.0 mg (1.95 mmol) of 3,4- dihydro-4,4-dimethyl-6-  
17 (2-(2-methyl-1,3-dioxolanyl))- 1(2H)-naphthalenone (Compound 100E) in 10  
18 ml of THF was added 496.2 mg (2.54 mmol; 2.54 ml of a 1 M solution in  
19 Et<sub>2</sub>O) of phenylmagnesium bromide. The resulting solution was heated to  
20 reflux for 8 hours, H<sub>2</sub>O was added and heating continued for 30 minutes.  
21 The THF was removed under reduced pressure and the aqueous residue was  
22 extracted with EtOAc. The combined organic layers were dried (MgSO<sub>4</sub>),  
23 concentrated under reduced pressure, and the title compound isolated from  
24 the residue by column chromatography (10% EtOAc - hexanes) as a colorless  
25 oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>) : δ 7.97 (1H, d, J = 1.8 Hz), 7.67 (1H, dd, J = 2.1,  
26 8.0 Hz), 7.34 (5H, m), 7.10 (1H, d, J = 8.1 Hz), 6.12 (1H, d, J = 4.6 Hz),  
27 2.59 (3H, s), 2.39 (2H, d, J = 4.8 Hz), 1.38 (6H, s).  
28 4-[3-oxo-3-(7,8-dihydro-5-phenyl-8,8-dimethyl-2-naphthalenyl)-1-propenyl]-  
29 benzoic acid (Compound 103)

1 To a solution of 115.0 mg (0.42 mmol) of 3,4- dihydro-1-phenyl-4,4-  
2 dimethyl-6-acetylnaphthalene (Compound 100H) and 65.0 mg (0.43 mmol) of  
3 4-formyl- benzoic acid in 5.0 ml EtOH and 1.0 ml THF, was added 120.0 mg  
4 (3.00 mmol; 3.0 ml of a 1 M aqueous solution) of NaOH. The resulting  
5 yellow solution was stirred at room temperature for 12 hours. The solution  
6 was acidified with 6% aqueous HCl and extracted with EtOAc. The  
7 combined organic layers were dried (MgSO<sub>4</sub>), concentrated under reduced  
8 pressure, and the title compounds was isolated by column chromatography  
9 (50% EtOAc - hexanes) as a pale yellow solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>) : δ 8.13  
10 (2H, d, J = 7.7 Hz), 8.04 (1H, s), 7.81 (1H, d, J = 15.5 Hz), 7.75 (3H, m),  
11 7.60 (1H, d, J = 15.5 Hz), 7.35 (5H, m), 7.14 (1H, d, J = 8.1 Hz), 6.15 (1H,  
12 t, J = 4.2 Hz), 2.41 (2H, d, J = 4.2 Hz), 1.41 (6H, s).

13 3-Fluoro-4-methoxythiophenol (Compound 200)

14 A flask containing Mg-turnings (651.9 mg, 26.8 g-atoms) was carefully  
15 flame dried under argon. Upon cooling to room temperature the flask was  
16 charged with 14.0 mL of THF, the mixture was heated to reflux temperature  
17 and 3-fluoro-4-methoxy-1-bromobenzene (5.00g ; 24.4 mmol) was slowly  
18 added while the mixture was at reflux. After 6 hours the formation of the  
19 Grignard reagent appeared complete. The resulting opaque orange solution  
20 was cooled to 0 °C and sulfur (781.8 mg, 24.4 g-atoms) was added in three  
21 portions over five minutes. After stirring overnight at room temperature the  
22 brown mixture was carefully poured into an ice 10% aqueous HCl mixture.  
23 Extraction with EtOAc was followed by washing the combined organic layers  
24 with H<sub>2</sub>O and saturated aqueous NaCl and drying over MgSO<sub>4</sub>. Removal of  
25 the solvents under reduced pressure and distillation (bulb-to-bulb) of the  
26 residue afforded 2.14 g (55%) of the title compound as a colorless oil, (bp  
27 90-100 °C / 5mm). <sup>1</sup>H NMR (300-MHz, CDCl<sub>3</sub>) δ: 7.06 (2H, m), 6.85 (1H, t,  
28 J = 8.6 Hz), 3.87 (3H, s), 3.43 (1H, s).

29 3-(3-fluoro-4-methoxy-phenylsulfanyl)-3-methyl-butyric acid (Compound 201)

1 A heavy-walled screw cap tube was charged with 3-methyl-2-butenic  
2 acid (1.23 g, 12.33 mmol), 3-fluoro-4-methoxy thiophenol (Compound 200,  
3 1.95 g, 12.33 mmol), and piperidine (314.8 mg, 3.70 mmol). This mixture was  
4 heated to 105 °C for 23 hours, cooled to room temperature and dissolved in  
5 EtOAc (100mL). The resulting solution was washed with 1M aqueous HCl,  
6 H<sub>2</sub>O, and saturated aqueous NaCl before being dried over Na<sub>2</sub>SO<sub>4</sub>.  
7 Concentration of the dry solution under reduced pressure afforded 3.22 g of  
8 a clear yellow oil which was used in the next reaction without further  
9 purification. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 7.32 (2H, m), 6.94 (1H, t, J =  
10 8.4 Hz), 3.92 (3H, s), 2.54 (2H, s), 1.41 (6H, s).

11 3-(3-fluoro-4-methoxy-phenylsulfanyl)-3-methyl-butyroyl chloride (Compound  
12 202)

13 To a solution of 3-(3-fluoro-4-methoxy-phenylsulfanyl)-3-methyl-  
14 butyric acid (Compound 201, 3.00 g, 11.6 mmol) in 40 mL of benzene at  
15 room temperature was added a solution of oxalyl chloride (2.21 g, 17.4 mmol)  
16 in 5 mL of benzene over 30 minutes. After 3 hours, the solution was washed  
17 with ice cold 5% aqueous NaOH (CAUTION: a large volume of gas is  
18 released during this procedure), followed by ice cold H<sub>2</sub>O, and finally  
19 saturated aqueous NaCl. The solution was dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated  
20 under reduced pressure to give 2.83 g of a clear yellow oil. This material was  
21 used without further purification in the next step. <sup>1</sup>H NMR (300 MHz,  
22 CDCl<sub>3</sub>) δ : 7.27 (2H, m), 6.96 (1H, t, J = 8.4 Hz), 3.93 (3H, s), 3.13 (2H, s),  
23 1.42 (6H, s).

24 6-Methoxy-7-fluoro-2,2-dimethyl-thiochroman-4-one (Compound 203)

25 To a solution of the acyl chloride (Compound 202, 2.80 g, 10.1 mmol)  
26 in 35 mL of CH<sub>2</sub>Cl<sub>2</sub> at 0 °C was added dropwise a solution of SnCl<sub>4</sub> (2.64 g,  
27 10.1 mmol) in 5 mL of CH<sub>2</sub>Cl<sub>2</sub>. After 2.5 hours the reaction was quenched  
28 by the slow addition of 20 mL H<sub>2</sub>O. The organic layer was washed with 1M  
29 aqueous HCl, 5% aqueous NaOH, H<sub>2</sub>O, and finally saturated aqueous NaCl

1 before being dried over  $\text{MgSO}_4$ . Concentration under reduced pressure  
2 afforded 1.90 g (78%) the title compound as a tan solid.  $^1\text{H}$  NMR (300 MHz,  
3  $\text{CDCl}_3$ )  $\delta$ : 7.72 (1H, d,  $J = 8.9$  Hz), 6.95 (1H, d,  $J = 11.0$  Hz), 3.91 (3H, s),  
4 2.85 (2H, s), 1.47 (6H, s).

5 6-Hydroxy-7-fluoro-2,2-dimethylthiochroman-4-one (Compound 204)

6 To a solution of 6-methoxy-7-fluoro-2,2-dimethyl-thiochroman-4-one  
7 (Compound 203, 1.75 g, 7.29 mmol) in 25 mL  $\text{CH}_2\text{Cl}_2$  cooled to  $-23^\circ\text{C}$  was  
8 added  $\text{BBr}_3$  (5.46 g, 21.8 mmol; 21.8 mL of a 1M solution in  $\text{CH}_2\text{Cl}_2$ ) over a  
9 10 minute period. After stirring for 23 hours at  $-230^\circ\text{C}$  the solution was  
10 warmed to  $0^\circ\text{C}$  for 3 hours, and then cooled to  $-78^\circ\text{C}$  and quenched by the  
11 slow addition of 25 mL of  $\text{H}_2\text{O}$ . Upon warming to room temperature the  
12 aqueous layer was extracted with  $\text{CH}_2\text{Cl}_2$  and the combined organic layers  
13 were washed with saturated aqueous  $\text{NaHCO}_3$ ,  $\text{H}_2\text{O}$ , and saturated aqueous  
14  $\text{NaCl}$  before being dried over  $\text{MgSO}_4$ . Removal of the solvents under  
15 reduced pressure, followed by column chromatography (10 to 20%  $\text{EtOAc}$  /  
16 hexanes) afforded 1.12 g (68%) of the title compound as a colorless solid.  $^1\text{H}$   
17 NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ : 7.82 (1H, d  $J = 9.3$  Hz), 6.97 (1H, d,  $J = 10.4$ —  
18 Hz), 5.54 (1H, s), 2.86 (2H, s), 1.46 (6H, s).

19 2,2-Dimethyl-4-oxo-7-fluoro-thiochroman-6-yl trifluoromethanesulfonate  
20 (Compound 205)

21 To a solution of 6-hydroxy-7-fluoro-2,2-dimethylthiochroman-4-one  
22 (Compound 204, 1.12 g, 4.94 mmol) in 25.0 mL of anhydrous pyridine at  $0^\circ\text{C}$   
23 was added trifluoromethanesulfonic anhydride (1.61 g, 5.68 mmol). After 18  
24 hours at  $0^\circ\text{C}$  the solution was concentrated and the residual oil dissolved in  
25  $\text{Et}_2\text{O}$ , washed with  $\text{H}_2\text{O}$  followed by saturated aqueous  $\text{NaCl}$ , and dried over  
26  $\text{MgSO}_4$ . Removal of the solvents under reduced pressure and column  
27 chromatography (5 to 8%  $\text{EtOAc}$  / hexanes) afforded 1.28 g (72%) of the  
28 title compound as a colorless solid.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ : 8.07 (1H,  
29 d,  $J = 7.8$  Hz), 7.13 (1H, d,  $J = 9.7$  Hz), 2.89 (2H, s), 1.50 (6H, s).

1 2,2-Dimethyl-6-trimethylsilylanylethynyl-7-fluoro-thiochroman-4-one  
2 (Compound 206)

3 A solution of 2,2-dimethyl-4-oxo-7-fluoro-thiochroman-6-yl  
4 trifluoromethanesulfonate (Compound 205, 1.28 g, 3.57 mmol) in 6.0 mL  
5 Et<sub>3</sub>N and 15.0 mL DMF was sparged with argon for 15 minutes. To this  
6 solution was added trimethylsilylacetylene (2.0 g, 20.4 mmol) and  
7 bis(triphenylphosphine)-palladium(II) chloride (100.0 mg, 0.14 mmol). The  
8 solution was heated to 95 °C for 6 hours, cooled to room temperature and  
9 stirred overnight. The solution was diluted with H<sub>2</sub>O and extracted with  
10 EtOAc. The combined organic layers were washed with H<sub>2</sub>O, saturated  
11 aqueous NaCl and dried over MgSO<sub>4</sub>. Concentration of the dry solution  
12 under reduced pressure and isolation of the product by column  
13 chromatography (3 to 5% EtOAc / hexanes) afforded 850.0 mg (78%) of the  
14 title compound as an orange solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 8.22 (1H,  
15 d, J = 7.5 Hz), 6.92 (1H, d, J = 9.3 Hz), 2.85 (2H, s), 1.46 (6H, s), 0.25 (9H,  
16 s).

17 6-Ethynyl-7-fluoro-2,2-dimethylthiochroman-4-one (A) (Compound 207), and  
18 6-Ethynyl-7-methoxy-2,2-dimethylthiochroman-4-one (B) (Compound 208)

19 A solution of 2,2-dimethyl-6-trimethylsilylanylethynyl-7-fluoro-  
20 thiochroman-4-one (Compound 206, 850.0 mg, 2.78mmol) and K<sub>2</sub>CO<sub>3</sub> (180.0  
21 mg, 1.30 mmol) in 20.0 mL MeOH was stirred overnight at room  
22 temperature. The solution was concentrated to one-third of the original  
23 volume, diluted with EtOAc, washed with H<sub>2</sub>O and saturated aqueous NaCl,  
24 and dried over MgSO<sub>4</sub>. Removal of the solvent under reduced pressure  
25 followed by column chromatography (5 to 10% EtOAc / hexanes) of the  
26 residual solid afforded 330.0 mg (51%) of Compound 207 as a yellow solid,  
27 and 217.0 mg (32%) of Compound 208 as a yellow solid. (Compound 207):  
28 <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 8.18 (1H, d, J = 7.5 Hz), 6.90 (1H, d, J = 8.3  
29 Hz), 3.28 (1H, s), 2.81 (2H, s), 1.42 (6H, s). (Compound 208); <sup>1</sup>H NMR (300

1 MHz, CDCl<sub>3</sub>)  $\delta$ : 8.19 (1H, s), 6.64 (1H, s), 3.90 (3H, s), 3.26 (1H, s), 2.80  
2 (2H, s), 1.44 (6H, s).

3 Ethyl 4-[(2,2-dimethyl-4-oxo-7-fluoro-thiochroman-6-yl)ethynyl]-benzoate  
4 (Compound 209)

5 A solution of 6-ethynyl-7-fluoro-2,2-dimethylthiochroman-4-one  
6 (Compound 207, 330.0 mg, 1.41 mmol) and ethyl 4-iodobenzoate (392 mg,  
7 1.41 mmol) in 10.0 mL Et<sub>3</sub>N was purged with argon for 15 minutes. To this  
8 solution was added bis(triphenylphosphine)-palladium(II) chloride (247 mg,  
9 0.35 mmol) and copper(I) iodide (67.0 mg, 0.35 mmol). After sparging for an  
10 additional 10 minutes with argon, the solution was stirred overnight at room  
11 temperature. The reaction mixture was diluted with EtOAc and filtered  
12 through a pad of Celite using an EtOAc wash. Concentration of the filtrate  
13 under reduced pressure, followed by column chromatography (3 to 5%  
14 EtOAc / hexanes) of the residual solid afforded 490.0 mg (91%) of the title  
15 compound as a yellow solid. <sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>)  $\delta$ : 8.31 (1H, d, J =  
16 7.6 Hz), 8.04 (2H, d, J = 8.3 Hz), 7.60 (2H, d, J = 8.3 Hz), 7.00 (1H, d, J =  
17 9.3 Hz), 4.40 (2H, q, J = 7.1 Hz), 2.89 (2H, s), 1.50 (6H, s), 1.41 (3H, t, J =  
18 7.1 Hz).

19 Ethyl 4-[(2,2-dimethyl-4-oxo-7-methoxy-thiochroman-6-yl)ethynyl]-benzoate  
20 (Compound 210)

21 A solution of 6-ethynyl-7-methoxy-2,2-dimethylthiochroman-4-one  
22 (Compound 208, 217.0 mg, 0.88 mmol) and ethyl 4-iodobenzoate (245.0 mg,  
23 0.89 mmol) in 10.0 mL Et<sub>3</sub>N and 2 mL THF was purged with argon for 15  
24 minutes. To this solution was added bis(triphenylphosphine)-palladium(II)  
25 chloride (154.0 mg, 0.22 mmol) and copper(I) iodide (42.0 mg, 0.22 mmol).  
26 After sparging for an additional 10 minutes with argon, the solution was  
27 stirred overnight at room temperature. The reaction mixture was diluted  
28 with EtOAc and filtered through a pad of Celite using an EtOAc wash.  
29 Concentration of the filtrate under reduced pressure, followed by column

1 chromatography (5 to 10% EtOAc / hexanes) of the residual solid afforded  
2 320.0 mg (92%) of the title compound as an orange solid. <sup>1</sup>H NMR (300  
3 MHz, CDCl<sub>3</sub>) δ: 8.29 (1H, s), 8.03 (2H, d, J = 8.4 Hz), 7.60 (2H, d, J = 8.4  
4 Hz), 6.70 (1H, s), 4.40 (2H, q, J = 7.1 Hz), 3.96 (3H, s), 2.86 (2H, s), 1.50  
5 (6H, s), 1.41 (3H, t, J = 7.1 Hz).

6 Ethyl 4-(2,2-dimethyl-4-trifluoromethanesulfonyloxy-7-fluoro-(2H)-  
7 thiochromen-6-ylethynyl)-benzoate (Compound 211)

8 A solution of sodium bis(trimethylsilyl)amide (256.7 mg, 1.44 mmol) in  
9 3.4 mL of THF was cooled to -78 °C and a solution of ethyl 4-(2,2-dimethyl-  
10 4-oxo-7-fluoro-thiochroman-6-ylethynyl)-benzoate (Compound 209, 460.0 mg,  
11 1.20 mmol) in 3.0 mL was added slowly. After 30 minutes a solution of 2-  
12 [N,N-bis(trifluoromethanesulfonyl)amino]-5-pyridine (518.0 mg, 1.32 mmol)  
13 in 2.0 mL of THF was added. After 5 minutes the solution was warmed to  
14 room temperature and stirred for 6 hours. The reaction was quenched by  
15 the addition of saturated aqueous NH<sub>4</sub>Cl and extracted with EtOAc. The  
16 combined organic layers were washed with 5% aqueous NaOH and H<sub>2</sub>O  
17 before being dried (MgSO<sub>4</sub>) and concentrated under reduced pressure. The  
18 title compound, 549.0 mg (89%), was isolated by column chromatography (3-  
19 5% EtOAc / hexanes) as a pale-yellow solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ:  
20 8.04 (2H, d, J = 8.4 Hz), 7.61 (2H, d, J = 8.4 Hz), 7.60 (1H, d, J = 6.7 Hz),  
21 7.08 (1H, d, J = 9.0 Hz), 5.88 (1H, s), 4.40 (2H, q, J = 7.1 Hz), 1.53 (6H, s),  
22 1.41 (3H, t, J = 7.1 Hz).

23 Ethyl 4-(2,2-dimethyl-4-trifluoromethanesulfonyloxy-7-methoxy-(2H)-  
24 thiochromen-6-ylethynyl)-benzoate (Compound 212)

25 A solution of sodium bis(trimethylsilyl)amide (155.9 mg, 0.85 mmol) in  
26 2.9 mL of THF was cooled to -78 °C and a solution of ethyl 4-(2,2-dimethyl-  
27 4-oxo-7-methoxy-thiochroman-6-ylethynyl)-benzoate (Compound 210, 280.0  
28 mg, 0.71 mmol) in 3.0 mL was added slowly. After 30 minutes a solution of  
29 2-[N,N-bis(trifluoromethanesulfonyl)amino]-5-pyridine (355.0 mg, 0.85 mmol)

1 in 2.0 mL of THF was added. After 5 minutes the solution was warmed to  
2 room temperature and stirred for 6 hours. The reaction was quenched by the  
3 addition of saturated aqueous  $\text{NH}_4\text{Cl}$  and extracted with EtOAc. The  
4 combined organic layers were washed with 5% aqueous NaOH and  $\text{H}_2\text{O}$   
5 before being dried ( $\text{MgSO}_4$ ) and concentrated under reduced pressure. The  
6 title compound, 300.0 mg (80%), was isolated by column chromatography  
7 (15% EtOAc / hexanes) as an orange solid.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ :  
8 8.02 (2H, d,  $J = 8.3$  Hz), 7.61 (2H, d,  $J = 8.3$  Hz), 7.57 (1H, s), 6.82 (1H, s),  
9 5.76 (1H, s), 4.38 (2H, q,  $J = 7.1$  Hz), 3.92 (3H, s), 1.51 (6H, s), 1.40 (3H, t,  
10  $J = 7.1$  Hz).

11 Ethyl 4-[[4-(4-methylphenyl)-2,2-dimethyl-7-fluoro-(2H)-thiochromen-6-yl]-  
12 ethynyl]-benzoate (Compound 213)

13 A solution of 4-methylbromobenzene (290.0 mg, 1.69 mmol) in 2.0 mL  
14 of THF was cooled to  $-78^\circ\text{C}$  and *tert*-butyllithium (217.2 mg, 3.39 mmol, 2.0  
15 mL of a 1.7M solution in pentane) was added to give a yellow solution.  
16 After 30 minutes a solution of  $\text{ZnCl}_2$  (409.0 mg, 3.00 mmol) in 4.0 mL THF  
17 was slowly added via cannula. The resulting solution was warmed to room  
18 temperature and transferred via cannula to a solution of ethyl 4-(2,2-  
19 dimethyl-4-trifluoromethanesulfonyloxy-7-fluoro-(2H)-thiochromen-6-  
20 ylethynyl)-benzoate (Compound 211, 158.0 mg, 0.31 mmol) and  
21 tetrakis(triphenylphosphine)palladium(0) (24.0 mg, 0.02 mmol) in 2.0 mL  
22 THF. This solution was heated to  $50^\circ\text{C}$  for 2 hours, cooled to room  
23 temperature, and the reaction quenched by the addition of saturated aqueous  
24  $\text{NH}_4\text{Cl}$ . This solution was extracted with EtOAc and the combined organic  
25 layers washed with  $\text{H}_2\text{O}$  and saturated aqueous NaCl before being dried  
26 ( $\text{MgSO}_4$ ) and concentrated under reduced pressure. The title compound,  
27 120.0 mg (86%), was isolated by column chromatography (3% EtOAc /  
28 hexanes).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ : 8.00 (2H, d,  $J = 8.3$  Hz), 7.54 (2H,  
29 d,  $J = 8.3$  Hz), 7.27-7.13 (6H, m), 5.79 (1H, s), 4.38 (2H, q,  $J = 7.1$  Hz), 2.42



1 (3H, s), 1.48 (6H, s), 1.40 (3H, t, J = 7.1 Hz).

2 Ethyl 4-[[4-(5-methyl-thiophen-2-yl)-2,2-dimethyl-7-fluoro-(2H)-thiochromen-  
3 6-yl]-ethynyl]-benzoate (Compound 214)

4 A solution of 2-methylthiophene (130.0 mg, 1.30 mmol) in 2.0 mL of  
5 THF was cooled to -78 °C and *n*-butyllithium (83.3 mg, 1.30 mmol, 0.84 ml of  
6 a 1.6M solution in hexanes) was added and the solution warmed to 0 °C  
7 during 1.5 hours. A solution of ZnCl<sub>2</sub> (341.0 mg, 2.50 mmol) in 3.0 mL THF  
8 was slowly added via cannula. The resulting solution was warmed to room  
9 temperature, stirred for 40 minutes, and transferred via cannula to a solution  
10 of ethyl 4-[(2,2-dimethyl-4-trifluoromethanesulfonyloxy-7-fluoro-(2H)-  
11 thiochromen-6-yl)ethynyl]-benzoate (Compound 211, 158.0 mg, 0.31  
12 mmol) and tetrakis(triphenylphosphine) palladium(0) (24.0 mg, 0.02 mmol) in  
13 2.0 mL THF. This solution was heated to 50 °C for 2 hours, cooled to room  
14 temperature, and the reaction quenched by the addition of saturated aqueous  
15 NH<sub>4</sub>Cl. The solution was extracted with EtOAc and the combined organic  
16 layers washed with H<sub>2</sub>O and saturated aqueous NaCl before being dried  
17 (MgSO<sub>4</sub>) and concentrated under reduced pressure. The title compound,  
18 70.0 mg (50%), was isolated by column chromatography (3% EtOAc /  
19 hexanes) as a waxy solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 8.01 (2H, d, J = 8.3  
20 Hz), 7.61 (1H, d, J = 7.5 Hz), 7.58 (2H, d, J = 8.3 Hz), 7.13 (1H, d, J = 7.2  
21 Hz), 6.82 (1H, d, J = 4.4 Hz), 6.73 (1H, d, J = 4.4 Hz), 5.96 (1H, s), 4.39  
22 (2H, q, J = 7.1 Hz), 2.52 (3H, s), 1.46 (6H, s), 1.41 (3H, t, J = 7.1 Hz).

23 Ethyl 4-[[4-(4-methylphenyl)-2,2-dimethyl-7-methoxy-(2H)-thiochromen-6-yl]-  
24 ethynyl]-benzoate (Compound 215)

25 A solution of 4-methylbromobenzene (499.0 mg, 2.92 mmol) in 2.0 mL  
26 of THF was cooled to -78 °C and *tert*-butyllithium (374.2 mg, 5.84 mmol, 3.4  
27 ml of a 1.7M solution in pentane) was added to give a yellow solution. After  
28 30 minutes a solution of ZnCl<sub>2</sub> (613.0 mg, 4.50 mmol) in 4.0 mL THF was  
29 slowly added via cannula. The resulting solution was warmed to room

1 temperature and transferred via cannula to a solution of ethyl 4-(2,2-  
2 dimethyl-4-trifluoromethanesulfonyloxy-7-methoxy-(2H)-thiochromen-6-  
3 ylethynyl)-benzoate (Compound 212, 285.0 mg, 0.54 mmol) and  
4 tetrakis(triphenylphosphine)palladium(0) (24.0 mg, 0.02 mmol) in 2.0 mL  
5 THF. This solution was heated to 50 °C for 2 hours, cooled to room  
6 temperature, and the reaction quenched by the addition of saturated aqueous  
7 NH<sub>4</sub>Cl. The solution was extracted with EtOAc and the combined organic  
8 layers were washed with H<sub>2</sub>O and saturated aqueous NaCl before being dried  
9 (MgSO<sub>4</sub>) and concentrated under reduced pressure. The title compound,  
10 200.0 mg (79%), was isolated by column chromatography (2-5% EtOAc /  
11 hexanes) as a colorless solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 7.98 (2H, d, J =  
12 8.3 Hz), 7.54 (2H, d, J = 8.3 Hz), 7.23-7.21 (5H, m), 5.71 (1H, s), 4.38 (2H,  
13 q, J = 7.1 Hz), 3.95 (3H, s), 2.41 (3H, s), 1.49 (6H, s), 1.40 (3H, t, J = 7.1  
14 Hz).

15 3-(4-methoxy-phenylsulfanyl)-3-methyl-butyric acid (Compound 216)

16 A heavy-walled screw cap tube was charged with 3-methyl-2-butenic  
17 acid (13.86g, 138.4 mmol), 4-methoxy thiophenol (20.0g, 138.4 mmol), and  
18 piperidine (3.45 g, 41.6 mmol). This mixture was heated to 105 °C for 32  
19 hours, cooled to room temperature and dissolved in EtOAc (700mL). The  
20 resulting solution was washed with 1M aqueous HCl, H<sub>2</sub>O, and saturated  
21 aqueous NaCl before being dried over Na<sub>2</sub>SO<sub>4</sub>. Concentration of the dry  
22 solution under reduced pressure afforded an oil which upon standing in the  
23 freezer provided a crystalline solid. The title compound was isolated as pale-  
24 yellow crystals by washing the crystalline solid with pentane. (27.33 g, 82%).  
25 <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 7.48 (2H, d, J = 9.0 Hz), 6.89 (2H, d, J = 8.9  
26 Hz), 3.83 (3H, s), 2.54 (2H, s), 1.40 (6H, s).

27 3-(4-methoxy-phenylsulfanyl)-3-methyl-butyryl chloride (Compound 217)

28 To a solution of 3-(4-methoxy-phenylsulfanyl)-3-methyl-butyric acid  
29 (Compound 216, 20.0 g, 83.2 mmol) in 250 mL of benzene at room

1 temperature was added a solution of oxalyl chloride (15.84g, 124.8 mmol) in  
2 10 mL of benzene over 30 minutes. After 4 hours the solution was washed  
3 with ice cold 5% aqueous NaOH (CAUTION: a large volume of gas is  
4 released during this procedure), followed by ice cold H<sub>2</sub>O, and finally  
5 saturated aqueous NaCl. The solution was dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated  
6 under reduced pressure to give a clear yellow oil. This material was used  
7 without further purification in the next step. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ :  
8 7.45 (2H, d, J = 8.8 Hz), 6.90 (2H, d, J = 8.8 Hz), 3.84 (3H, s), 3.12 (2H, s),  
9 1.41 (6H, s).

10 6-Methoxy-2,2-dimethyl-thiochroman-4-one (Compound 218)

11 To a solution of the acyl chloride (Compound 217, 21.5g, 83.2 mmol)  
12 in 250 mL of CH<sub>2</sub>Cl<sub>2</sub> at 0 °C was added dropwise a solution of SnCl<sub>4</sub> (21.7g,  
13 83.2 mmol) in 30 mL of CH<sub>2</sub>Cl<sub>2</sub>. After 2 hours the reaction was quenched by  
14 slow addition of 150 mL H<sub>2</sub>O. The organic layer was washed with 1M  
15 aqueous HCl, 5% aqueous NaOH, H<sub>2</sub>O, and finally saturated aqueous NaCl  
16 before being dried over MgSO<sub>4</sub>. Concentration under reduced pressure and  
17 vacuum distillation of the residual oil (Bulb-to-bulb, 125-135 °C, 5 mm/Hg)  
18 afforded 14.48 g (78%) of the title compound as a pale-yellow oil. <sup>1</sup>H NMR  
19 (300 MHz, CDCl<sub>3</sub>) δ: 7.62 (1H, d, J = 2.9 Hz), 7.14 (1H, d, J = 8.6 Hz), 7.03  
20 (1H, dd, J = 2.8, 8.3 Hz), 3.83 (3H, s), 2.87 (2H, s), 1.46 (6H, s).

21 6-Hydroxy-2,2-dimethylthiochroman-4-one (Compound 219)

22 To a solution of 6-methoxy-2,2-dimethyl-thiochroman-4-one  
23 (Compound 218, 6.0 g, 27 mmol) in 50 mL CH<sub>2</sub>Cl<sub>2</sub> cooled to -23 °C was  
24 added BBr<sub>3</sub> (20.0 g, 80.0 mmol; 80.0 mL of a 1M solution in CH<sub>2</sub>Cl<sub>2</sub>) over a  
25 20 minute period. After stirring for 5 hours at -23 °C the solution was cooled  
26 to -78 °C and quenched by the slow addition of 50 mL of H<sub>2</sub>O. Upon  
27 warming to room-temperature the aqueous layer was extracted with CH<sub>2</sub>Cl<sub>2</sub>  
28 and the combined organic layers were washed with saturated aqueous  
29 NaHCO<sub>3</sub>, H<sub>2</sub>O, and saturated aqueous NaCl before being dried over MgSO<sub>4</sub>.

1 Removal of the solvents under reduced pressure gave a green-brown solid  
2 which upon recrystallization (Et<sub>2</sub>O / hexanes) afforded 2.25 g (40%) of the  
3 title compound as a light brown solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 7.63  
4 (1H, d, J = 2.8 Hz), 7.15 (1H, d, J = 8.5 Hz), 7.01 (1H, dd, J = 2.8, 8.5 Hz),  
5 2.87 (2H, s), 1.46 (6H, s).

6 2,2-Dimethyl-4-oxo-thiochroman-6-yl trifluoromethanesulfonate (Compound  
7 220)

8 To a solution of 6-hydroxy-2,2-dimethylthiochroman-4-one (Compound  
9 219, 165.0 mg, 0.79 mmol) in 5.0 mL of anhydrous pyridine at 0 °C was  
10 added trifluoromethanesulfonic anhydride (245.0 mg, 0.87 mmol). After 4  
11 hours at 0 °C the solution was concentrated and the residual oil dissolved in  
12 Et<sub>2</sub>O, washed with H<sub>2</sub>O followed by saturated aqueous NaCl, and dried over  
13 MgSO<sub>4</sub>. Removal of the solvents under reduced pressure and column  
14 chromatography (5% EtOAc / hexanes) afforded 126.0 mg (47%) of the title  
15 compound as a colorless solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 7.97 (1H, s),  
16 7.32 (2H, s), 2.90 (2H, s), 1.49 (6H, s).

17 2,2-Dimethyl-6-trimethylsilyl-ethynyl-thiochroman-4-one (Compound 221)

18 A solution of 2,2-dimethyl-4-oxo-thiochroman-6-yl  
19 trifluoromethanesulfonate (Compound 220, 2.88 g, 8.50 mmol) in 10 mL Et<sub>3</sub>N  
20 and 20.0 mL DMF was sparged with argon for 10 minutes. To this solution  
21 was added trimethylsilylacetylene (4.15 g, 42.0 mmol) and  
22 bis(triphenylphosphine)-palladium(II) chloride (298.0 mg, 0.425 mmol). The  
23 solution was heated to 95 °C for 5 hours, cooled to room temperature, and  
24 diluted with H<sub>2</sub>O. Extraction with EtOAc was followed by washing the  
25 combined organic layers with H<sub>2</sub>O and saturated aqueous NaCl and drying  
26 over MgSO<sub>4</sub>. Concentration of the dry solution under reduced pressure and  
27 isolation of the product by column chromatography (3% EtOAc / hexanes)  
28 afforded 2.23 g (91%) of the title compound as an orange oil. <sup>1</sup>H NMR (300  
29 MHz, CDCl<sub>3</sub>) δ: 8.18 (1H, d, J = 1.9 Hz), 7.34 (1H, dd, J = 1.9, 8.1 Hz), 7.15

1 (1H, d, J = 8.1 Hz), 2.85 (2H, s), 1.45 (6H, s), 0.23 (9H, s).

2 6-Ethynyl-2,2-dimethylthiochroman-4-one (Compound 222)

3 A solution of 2,2-dimethyl-6-trimethylsilanylethynyl-thiochroman-4-one  
4 (Compound 221, 110.0 mg, 0.38 mmol) and K<sub>2</sub>CO<sub>3</sub> (40.0 mg, 0.29 mmol) in  
5 10.0 mL MeOH was stirred overnight at room temperature. The solution  
6 was diluted with H<sub>2</sub>O and extracted with Et<sub>2</sub>O. The combined organic layers  
7 were washed with H<sub>2</sub>O and saturated aqueous NaCl and dried over MgSO<sub>4</sub>.  
8 Removal of the solvent under reduced pressure afforded 81 mg (99%) of the  
9 title compound as an orange oil. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 8.20 (1H, d, J  
10 = 1.9 Hz), 7.46 (1H, dd, J = 1.9, 8.1 Hz), 7.18 (1H, d, J = 8.1 Hz), 3.08 (1H,  
11 s), 2.86 (2H, s), 1.46 (6H, s).

12 Ethyl 4-[(2,2-dimethyl-4-oxo-thiochroman-6-yl)ethynyl]-benzoate (Compound  
13 223)

14 A solution of 6-ethynyl-2,2-dimethylthiochroman-4-one (Compound  
15 222, 82.0 mg, 0.38 mmol) and ethyl 4-iodobenzoate (104.9 mg, 0.38 mmol) in  
16 5.0 mL Et<sub>3</sub>N was purged with argon for 10 minutes. To this solution were  
17 added bis(triphenylphosphine)-palladium(II)-chloride (88.0 mg, 0.12 mmol)  
18 and copper(I) iodide (22.9 mg, 0.12 mmol). After sparging for an additional  
19 5 minutes with argon, the solution was stirred overnight at room temperature.  
20 The reaction mixture was filtered through a pad of Celite using an Et<sub>2</sub>O  
21 wash. Concentration of the filtrate under reduced pressure, followed by  
22 column chromatography of the residual solid, afforded 100 mg (72%) of the  
23 title compound as a yellow solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 8.25 (1H, d,  
24 J = 1.8 Hz), 8.00 (2H, d, J = 8.4 Hz), 7.55 (2H, d, J = 8.4 Hz), 7.53 (1H, dd,  
25 J = 1.8, 8.2 Hz), 7.21 (1H, d, J = 8.2 Hz), 4.37 (2H, q, J = 7.1 Hz), 2.88  
26 (2H, s), 1.47 (6H, s), 1.39 (3H, t, J = 7.1 Hz).

27 Ethyl 4-[(2,2-dimethyl-4-trifluoromethanesulfonyloxy-(2H)-thiochromen-6-  
28 yl)ethynyl]-benzoate (Compound 224)

29 A solution of sodium bis(trimethylsilyl)amide (1.12 g, 6.13 mmol) in

1 16.2 mL of THF was cooled to -78 °C and a solution of ethyl 4-(2,2-dimethyl-  
2 4-oxo-thiochroman-6-ylethynyl)-benzoate (Compound 223, 1.86g, 5.10 mmol)  
3 in 15.0 mL was added slowly. After 30 minutes a solution of 2-[N,N-  
4 bis(trifluoromethanesulfonyl)amino]-5-pyridine (2.40 g, 6.13 mmol) in 10 mL  
5 of THF was added. After 5 minutes the solution was warmed to room  
6 temperature and stirred overnight. The reaction was quenched by the  
7 addition of saturated aqueous NH<sub>4</sub>Cl and extracted with EtOAc. The  
8 combined organic layers were washed with 5% aqueous NaOH and H<sub>2</sub>O  
9 before being dried (MgSO<sub>4</sub>) and concentrated under reduced pressure. The  
10 title compound, 1.53 g (61%), was isolated by column chromatography (2%  
11 EtOAc / hexanes) as a yellow solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 8.03 (2H,  
12 d, J = 8.4 Hz), 7.61 (1H, d, J = 1.8 Hz), 7.59 (2H, d, J = 8.4 Hz), 7.41 (1H,  
13 dd, J = 1.8, 8.1Hz), 7.29 (1H, d, J = 8.1 Hz), 5.91 (1H, s), 4.39 (2H, q, J =  
14 7.1 Hz), 1.53 (6H, s), 1.41 (3H, t, J = 7.1 Hz).

15 Ethyl 4-[[4-phenyl-2,2-dimethyl-(2H)-thiochromen-6-yl]-ethynyl]-benzoate  
16 (Compound 225)

17 A solution of bromobenzene (190.0 mg, 1.18 mmol) in 2.0 mL of THF  
18 was cooled to -78 °C and *tert*-butyllithium (151.2 mg, 2.36 mmol, 1.4 mL of a  
19 1.7M solution in pentane) was added to give a yellow solution. After 30  
20 minutes a solution of ZnCl<sub>2</sub> (225.0 mg, 1.4 mmol) in 4.0 mL THF was slowly  
21 added via cannula. The resulting solution was warmed to room temperature  
22 and transferred via cannula to a solution of ethyl 4-(2,2-dimethyl-4-  
23 trifluoromethanesulfonyloxy-(2H)-thiochromen-6-ylethynyl)-benzoate  
24 (Compound 224, 200.0 mg, 0.40 mmol) and  
25 tetrakis(triphenylphosphine)palladium(0) (24.0 mg, 0.02 mmol) in 2.0 mL  
26 THF. This solution was heated to 50 °C for 2 hours, cooled to room  
27 temperature, and the reaction quenched by the addition of saturated aqueous  
28 NH<sub>4</sub>Cl. The solution was extracted with EtOAc and the combined organic  
29 layers were washed with H<sub>2</sub>O and saturated aqueous NaCl before being dried

1 (MgSO<sub>4</sub>) and concentrated under reduced pressure. The title compound,  
2 155.0 mg (91%), was isolated by column chromatography (5% EtOAc /  
3 hexanes) as a colorless solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 7.99 (2H, d, J =  
4 8.4 Hz), 7.52 (2H, d, J = 8.4 Hz), 7.42-7.24 (8H, m), 5.78 (1H, s), 4.38 (2H,  
5 q, J = 7.1 Hz), 1.50 (6H, s), 1.40 (3H, t, J = 7.1 Hz).

6 Ethyl 4-[[4-(4-methylphenyl)-2,2-dimethyl-(2H)-thiochromen-6-yl]-ethynyl]-  
7 benzoate (Compound 226)

8 A solution of 4-methylbromobenzene (203.0 mg, 1.15 mmol) in 2.0 mL  
9 of THF was cooled to -78 °C and *tert*-butyllithium (147.4 mg, 2.30 mmol, 1.4  
10 ml of a 1.7M solution in pentane) was added to give a yellow solution. After  
11 30 minutes a solution of ZnCl<sub>2</sub> (219.4 mg, 1.4 mmol) in 4.0 mL THF was  
12 slowly added via cannula. The resulting solution was warmed to room  
13 temperature and transferred via cannula to a solution of ethyl 4-(2,2-  
14 dimethyl-4-trifluoromethanesulfonyloxy-(2H)-thiochromen-6-ylethynyl)-  
15 benzoate (Compound 224, 230.0 mg, 0.46 mmol) and  
16 tetrakis(triphenylphosphine)palladium(0) (24.0 mg, 0.02 mmol) in 2.0 mL  
17 THF. This solution was heated to 50 °C for 2-hours, cooled to room  
18 temperature, and the reaction quenched by the addition of saturated aqueous  
19 NH<sub>4</sub>Cl. The solution was extracted with EtOAc and the combined organic  
20 layers were washed with H<sub>2</sub>O and saturated aqueous NaCl before being dried  
21 (MgSO<sub>4</sub>) and concentrated under reduced pressure. The title compound,  
22 165.0 mg (82%), was isolated by column chromatography (5% EtOAc /  
23 hexanes) as a colorless solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 7.98 (2H, d, J =  
24 8.4 Hz), 7.51 (2H, d, J = 8.4 Hz), 7.35-7.21 (7H, m), 5.84 (1H, s), 4.38 (2H,  
25 q, J = 7.1 Hz), 2.42 (3H, s), 1.48 (6H, s), 1.40 (3H, s).

26 Ethyl 4-[[4-(4-ethylphenyl)-2,2-dimethyl-(2H)-thiochromen-6-yl]-ethynyl]-  
27 benzoate (Compound 227)

28 A solution of 4-ethylbromobenzene (670.9 mg, 3.63 mmol) in 4.0 mL  
29 of THF was cooled to -78 °C and *tert*-butyllithium (464.5 mg, 7.25 mmol, 4.26

1 mL of a 1.7M solution in pentane) was added to give a yellow solution.  
2 After 30 minutes a solution of  $\text{ZnCl}_2$  (658.7 mg, 4.83 mmol) in 8.0 mL THF  
3 was slowly added via cannula. The resulting solution was warmed to room  
4 temperature and transferred via cannula to a solution of ethyl 4-(2,2-  
5 dimethyl-4-trifluoromethanesulfonyloxy-(2H)-thiochromen-6-ylethynyl)-  
6 benzoate (Compound 224, 1.20 g, 2.42 mmol) and  
7 tetrakis(triphenylphosphine)palladium(0) (111.7 mg, 0.097 mmol) in 8.0 mL  
8 THF. This solution was heated to 50 °C for 1 hour, cooled to room  
9 temperature, and the reaction quenched by the addition of saturated aqueous  
10  $\text{NH}_4\text{Cl}$ . The solution was extracted with EtOAc and the combined organic  
11 layers were washed with  $\text{H}_2\text{O}$  and saturated aqueous NaCl before being dried  
12 ( $\text{MgSO}_4$ ) and concentrated under reduced pressure. The title compounds  
13 was isolated by column chromatography (5% EtOAc / hexanes) as a colorless  
14 oil.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ : 7.99 (2H, d,  $J$  = 8.2 Hz), 7.52 (2H, d,  $J$  =  
15 8.4 Hz), 7.40 (5H, m), 7.35 (2H, m), 5.85 (1H, s), 4.38 (2H, q,  $J$  = 7.1 Hz),  
16 2.72 (2H, q,  $J$  = 7.6 Hz), 1.48 (6H, s), 1.40 (3H, t,  $J$  = 7.1 Hz), 1.30 (3H, t,  $J$   
17 = 7.6 Hz).

18 Ethyl 4-[[4-(4-isopropylphenyl)-2,2-dimethyl-(2H)-thiochromen-6-yl]-ethynyl]-  
19 benzoate (Compound 228)

20 A solution of 4-isopropylbromobenzene (161.0 mg, 0.81 mmol) in 2.0  
21 mL of THF was cooled to -78 °C and *tert*-butyllithium (103.8 mg, 1.62 mmol,  
22 0.95 ml of a 1.7M solution in pentane) was added to give a yellow solution.  
23 After 30 minutes a solution of  $\text{ZnCl}_2$  (175.5 mg, 1.3 mmol) in 4.0 mL THF  
24 was slowly added via cannula. The resulting solution was warmed to room  
25 temperature and transferred via cannula to a solution of ethyl 4-(2,2-  
26 dimethyl-4-trifluoromethanesulfonyloxy-(2H)-thiochromen-6-ylethynyl)-  
27 benzoate (Compound 224, 160.0 mg, 0.32 mmol) and  
28 tetrakis(triphenylphosphine) palladium(0) (24.0 mg, 0.02 mmol) in 2.0 mL  
29 THF. This solution was heated to 50 °C for 2 hours, cooled to room



1 temperature, and the reaction quenched by the addition of saturated aqueous  
2  $\text{NH}_4\text{Cl}$ . This solution was extracted with EtOAc and the combined organic  
3 layers were washed with  $\text{H}_2\text{O}$  and saturated aqueous NaCl before being dried  
4 ( $\text{MgSO}_4$ ) and concentrated under reduced pressure. The title compound,  
5 128.0 mg (85%), was isolated by column chromatography (5% EtOAc /  
6 hexanes) as a colorless oil.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ : 7.99 (2H, d,  $J$  =  
7 8.4 Hz), 7.53 (2H, d,  $J$  = 8.1 Hz), 7.38-7.22 (7H, m), 5.86 (1H, s), 4.38 (2H,  
8 q,  $J$  = 7.1 Hz), 2.98 (1H, hept, 7.0 Hz), 1.49 (6H, s), 1.43 (3H, t,  $J$  = 7.1 Hz),  
9 1.32 (6H, d,  $J$  = 7.0 Hz).

10 Ethyl 4-[[4-(4-*tert*-butylphenyl)-2,2-dimethyl-(2H)-thiochromen-6-yl]-ethynyl]-  
11 benzoate (Compound 229)

12 A solution of 4-*tert*-butylbromobenzene (149.0 mg, 0.70 mmol) in 2.0  
13 mL of THF was cooled to -78 °C and *tert*-butyllithium (92.6 mg, 1.44 mmol,  
14 0.85 ml of a 1.7M solution in pentane) was added to give a yellow solution.  
15 After 30 minutes a solution of  $\text{ZnCl}_2$  (133.0 mg, 0.98 mmol) in 4.0 mL THF  
16 was slowly added via cannula. The resulting solution was warmed to room  
17 temperature and transferred via cannula to a solution of ethyl 4-(2,2-  
18 dimethyl-4-trifluoromethanesulfonyloxy-(2H)-thiochromen-6-ylethynyl)-  
19 benzoate (Compound 224, 140.0 mg, 0.28 mmol) and  
20 tetrakis(triphenylphosphine) palladium(0) (24.0 mg, 0.02 mmol) in 2.0 mL  
21 THF. This solution was heated to 50 °C for 2 hours, cooled to room  
22 temperature, and the reaction quenched by the addition of saturated aqueous  
23  $\text{NH}_4\text{Cl}$ . The solution was extracted with EtOAc and the combined organic  
24 layers were washed with  $\text{H}_2\text{O}$  and saturated aqueous NaCl before being dried  
25 ( $\text{MgSO}_4$ ) and concentrated under reduced pressure. The title compound,  
26 94.0 mg (70%), was isolated by column chromatography (5% EtOAc /  
27 hexanes) as a colorless solid.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ : 7.98 (2H, d,  $J$  =  
28 8.3 Hz), 7.52 (2H, d,  $J$  = 8.3 Hz), 7.43-7.22 (7H, m), 5.86 (1H, s), 4.38 (2H,  
29 q,  $J$  = 7.1 Hz), 1.47 (6H, s), 1.40 (3H, t,  $J$  = 7.1 Hz), 1.38 (9H, s).

1 Ethyl 4-[[4-(5-methyl-thiophen-2-yl)-2,2-dimethyl-(2H)-thiochromen-6-yl]-  
2 ethynyl]-benzoate (Compound 230)

3 A solution of 2-methylthiophene (100.0 mg, 1.00 mmol) in 2.0 mL of  
4 THF was cooled to -78 °C and *n*-butyllithium (64.0 mg, 1.00 mmol, 0.63 ml of  
5 a 1.6M solution in hexanes) was added and the solution warmed to 0 °C  
6 during 1.5 hours. A solution of ZnCl<sub>2</sub> (218.0 mg, 1.60 mmol) in 3.0 mL THF  
7 was slowly added via cannula. The resulting solution was warmed to room  
8 temperature, stirred for 40 minutes, and transferred via cannula to a solution  
9 of ethyl 4-[(2,2-dimethyl-4-trifluoromethanesulfonyloxy-(2H)-thiochromen-6-  
10 yl)ethynyl]-benzoate (Compound 224, 200.0 mg, 0.40 mmol) and  
11 tetrakis(triphenylphosphine) palladium(0) (24.0 mg, 0.02 mmol) in 2.0 mL  
12 THF. This solution was heated to 50 °C for 2 hours, cooled to room  
13 temperature, and the reaction quenched by the addition of saturated aqueous  
14 NH<sub>4</sub>Cl. The solution was extracted with EtOAc and the combined organic  
15 layers were washed with H<sub>2</sub>O and saturated aqueous NaCl before being dried  
16 (MgSO<sub>4</sub>) and concentrated under reduced pressure. The title compound,  
17 170.0 mg (96%), was isolated by column chromatography (5% EtOAc /  
18 hexanes) as a pale-yellow solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 8.01 (2H, d, J  
19 = 8.3 Hz), 7.63 (1H, s), 7.55 (2H, d, J = 8.3 Hz), 7.35 (2H, s), 6.82 (1H, d, J  
20 = 3.5 Hz), 6.72 (1H, m), 6.00 (1H, s), 4.38 (2H, q, J = 7.1 Hz), 2.52 (3H, s),  
21 1.46 (6H, s), 1.40 (2H, t, J = 7.1 Hz).

22 Ethyl 4-[[4-(5-ethyl-thiophen-2-yl)-2,2-dimethyl-(2H)-thiochromen-6-yl]-  
23 ethynyl]-benzoate (Compound 231)

24 A solution of 2-ethylthiophene (112.0 mg, 1.00 mmol) in 2.0 mL of  
25 THF was cooled to -78 °C and *n*-butyllithium (64.0 mg, 1.00 mmol, 0.63 ml of  
26 a 1.6M solution in hexanes) was added and the solution warmed to 0 °C  
27 during 1.5 hours. A solution of ZnCl<sub>2</sub> (218.0 mg, 1.60 mmol) in 3.0 mL THF  
28 was slowly added via cannula. The resulting solution was warmed to room  
29 temperature, stirred for 40 minutes, and transferred via cannula to a solution

1 of ethyl 4-[(2,2-dimethyl-4-trifluoromethanesulfonyloxy-(2H)-thiochromen-6-  
2 yl)ethynyl]-benzoate (Compound 224, 200.0 mg, 0.40 mmol) and  
3 tetrakis(triphenylphosphine) palladium(0) (24.0 mg, 0.02 mmol) in 2.0 mL  
4 THF. This solution was heated to 50 °C for 2 hours, cooled to room  
5 temperature, and the reaction quenched by the addition of saturated aqueous  
6 NH<sub>4</sub>Cl. The solution was extracted with EtOAc and the combined organic  
7 layers were washed with H<sub>2</sub>O and saturated aqueous NaCl before being dried  
8 (MgSO<sub>4</sub>) and concentrated under reduced pressure. The title compound,  
9 29.0 mg (16%), was isolated by HPLC (1.25% EtOAc / hexanes) as a pale-  
10 yellow solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 8.01 (2H, d, J = 8.4 Hz), 7.65  
11 (1H, s), 7.55 (2H, d, J = 8.4 Hz), 7.35 (2H, s), 6.84 (1H, d, J = 3.5 Hz), 6.77  
12 (1H, m), 6.02 (1H, s), 4.39 (2H, q, J = 7.1 Hz), 2.88 (2H, q, J = 7.6 Hz),  
13 1.46 (6H, s), 1.41 (2H, t, J = 7.1 Hz), 1.35 (2H, t, J = 7.6 Hz),  
14 Ethyl 4-[[4-(5-*tert*-butyl-thiophen-2-yl)-2,2-dimethyl-(2H)-thiochromen-6-yl]-  
15 ethynyl]-benzoate (Compound 232)

16 A solution of 2-*tert*-butylthiophene (105.0 mg, 0.75 mmol) in 2.0 mL of  
17 THF was cooled to -78 °C and *n*-butyllithium (48.1 mg, 0.75 mmol, 0.47 ml of  
18 a 1.6M solution in hexanes) was added and the solution warmed to 0 °C  
19 during 1.5 hours. A solution of ZnCl<sub>2</sub> (163.2 mg, 1.20 mmol) in 3.0 mL THF  
20 was slowly added via cannula. The resulting solution was warmed to room  
21 temperature, stirred for 40 minutes, and transferred via cannula to a solution  
22 of ethyl 4-[(2,2-dimethyl-4-trifluoromethanesulfonyloxy-(2H)-thiochromen-6-  
23 yl)ethynyl]-benzoate (Compound 224, 200.0 mg, 0.40 mmol) and  
24 tetrakis(triphenylphosphine) palladium(0) (20.0 mg, 0.02 mmol) in 2.0 mL  
25 THF. This solution was heated to 50 °C for 2 hours, cooled to room  
26 temperature, and the reaction quenched by the addition of saturated aqueous  
27 NH<sub>4</sub>Cl. The solution was extracted with EtOAc and the combined organic  
28 layers were washed with H<sub>2</sub>O and saturated aqueous NaCl before being dried  
29 (MgSO<sub>4</sub>) and concentrated under reduced pressure. The title compound,

1 110.0 mg (76%), was isolated by column chromatography (5% EtOAc /  
2 hexanes) as a colorless solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 8.01 (2H, d, J =  
3 8.3 Hz), 7.68 (1H, s), 7.56 (2H, d, J = 8.3 Hz), 7.36 (2H, m), 6.82 (2H, m),  
4 6.04 (1H, s), 4.39 (2H, q, J = 7.1 Hz), 1.46 (6H, s), 1.43 (9H, s), 1.41 (2H, t,  
5 J = 7.1 Hz).

6 Ethyl 4-[[4-(6-methyl-pyridin-3-yl)-2,2-dimethyl-(2H)-thiochromen-6-yl]-  
7 ethynyl]-benzoate (Compound 233)

8 A solution of 4-bromo-2-methylpyridine (220.0 mg, 1.28 mmol) in 2.0  
9 mL of THF was cooled to -78 °C and *tert*-butyllithium (164.0 mg, 2.56 mmol,  
10 1.5 ml of a 1.7M solution in pentane) was added to give a red-orange  
11 solution. After 30 minutes a solution of ZnCl<sub>2</sub> (348.0 mg, 2.56 mmol) in 4.0  
12 mL THF was slowly added via cannula. The resulting solution was warmed  
13 to room temperature, stirred for 40 minutes, and transferred via cannula to a  
14 solution of ethyl 4-(2,2-dimethyl-4-trifluoromethanesulfonyloxy-(2H)-  
15 thiochromen-6-ylethynyl)-benzoate (Compound 224, 172.0 mg, 0.35 mmol)  
16 and tetrakis(triphenylphosphine) palladium(0) (24.0 mg, 0.02 mmol) in 2.0  
17 mL THF. This solution was heated to 50 °C for 2 hours, cooled to room  
18 temperature, and the reaction quenched by the addition of saturated aqueous  
19 NH<sub>4</sub>Cl. The solution was extracted with EtOAc and the combined organic  
20 layers were washed with H<sub>2</sub>O and saturated aqueous NaCl before being dried  
21 (MgSO<sub>4</sub>) and concentrated under reduced pressure. The title compound,  
22 109.0 mg (72%) was isolated by column chromatography (5% EtOAc /  
23 hexanes) as a pale-yellow solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 8.45 (1H, d, J  
24 = 2.0 Hz), 8.00 (2H, d, J = 8.5 Hz), 7.52 (2H, d, J = 8.5 Hz), 7.49 (1H, dd, J  
25 = 2.3, 8.1 Hz), 7.36 (2H, m), 7.20 (1H, d, J = 8.1 Hz), 7.16 (1H, d, J = 1.5  
26 Hz), 5.86 (1H, s), 4.38 (2H, q, J = 7.1 Hz), 2.63 (3H, s), 1.50 (6H, s), 1.40  
27 (3H, t, J = 7.1 Hz).

28 Ethyl 4-[(2,2-dimethyl-4-oxo-thiochroman-6-yl)ethynyl]-2-fluorobenzoate  
29 (Compound 234)

1 A solution of 6-ethynyl-2,2-dimethylthiochroman-4-one (Compound  
2 222, 1.37g, 6.34 mmol) and ethyl 2-fluoro-4-iodobenzoate (1.86g, 6.34mmol) in  
3 40.0 mL Et<sub>3</sub>N was purged with argon for 20 minutes. To this solution was  
4 added bis(triphenylphosphine)-palladium(II) chloride (1.05 g, 1.5 mmol) and  
5 copper(I) iodide (286.0 mg, 1.5 mmol). After sparging for an additional 10  
6 minutes with argon, the solution was stirred overnight at room temperature.  
7 The reaction mixture was filtered through a pad of Celite using an Et<sub>2</sub>O  
8 wash. Concentration of the filtrate under reduced pressure, followed by  
9 column chromatography (5% EtOAc / hexanes) of the residual solid afforded  
10 1.88 g (78%) the title compound as a yellow solid. <sup>1</sup>H NMR (300 MHz,  
11 CDCl<sub>3</sub>) δ: 8.27 (1H, d, J = 1.9 Hz), 7.92 (1H, d, J = 7.8 Hz), 7.52 (1H, dd, J  
12 = 1.9, 8.2 Hz), 7.36-7.24 (3H, m), 4.41 (2H, q, J = 7.1 Hz), 2.90 (2H, s), 1.50  
13 (6H, s), 1.41 (3H, t, J = 7.1 Hz).

14 Ethyl 4-(2,2-dimethyl-4-trifluoromethanesulfonyloxy-(2H)-thiochromen-6-  
15 ylethynyl)-2-fluorobenzoate (Compound 235)

16 A solution of sodium bis(trimethylsilyl)amide (0.82g, 5.90 mmol) in  
17 16.2 mL of THF was cooled to -78 °C and a solution of ethyl 4-[(2,2-  
18 dimethyl-4-oxo-thiochroman-6-yl)ethynyl]-2-fluorobenzoate (Compound 234,  
19 1.88g, 4.91 mmol) in 15.0 mL was added slowly. After 30 minutes a solution  
20 of 2-[N,N-bis(trifluoromethanesulfonyl)amino]-5-pyridine (2.32 g, 5.90 mmol)  
21 in 10 mL of THF was added. After 5 minutes the solution was warmed to  
22 room temperature and stirred overnight. The reaction was quenched by the  
23 addition of saturated aqueous NH<sub>4</sub>Cl and extracted with EtOAc. The  
24 combined organic layers were washed with 5% aqueous NaOH and H<sub>2</sub>O  
25 before being dried (MgSO<sub>4</sub>) and concentrated under reduced pressure. The  
26 title compound, 1.80 g (71%), was isolated by column chromatography (5%  
27 EtOAc / hexanes) as a yellow solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 7.93 (1H,  
28 d, J = 7.8 Hz), 7.61 (1H, d, J = 1.6 Hz), 7.43-7.27 (4H, m), 5.92 (1H, s), 4.41  
29 (2H, q, J = 7.1 Hz), 1.53 (6H, s), 1.42 (3H, t, J = 7.1 Hz).

1 Ethyl 4-[[4-(4-methylphenyl)-2,2-dimethyl-(2H)-thiochromen-6-yl]-ethynyl]-2-  
2 fluorobenzoate (Compound 236)

3 A solution of 4-methylbromobenzene (280.0 mg, 1.64 mmol) in 2.0 mL  
4 of THF was cooled to -78 °C and *tert*-butyllithium (209.5 mg, 3.27 mmol, 1.9  
5 mL of a 1.7M solution in pentane) was added to give a yellow solution.  
6 After 30 minutes a solution of ZnCl<sub>2</sub> (680.0 mg, 5.0 mmol) in 4.0 mL THF  
7 was slowly added via cannula. The resulting solution was warmed to room  
8 temperature and transferred via cannula to a solution of ethyl 4-(2,2-  
9 dimethyl-4-trifluoromethanesulfonyloxy-(2H)-thiochromen-6-ylethynyl)-2-  
10 fluorobenzoate (Compound 234, 200.0 mg, 0.39 mmol) and  
11 tetrakis(triphenylphosphine)palladium(0) (24.0 mg, 0.02 mmol) in 2.0 mL  
12 THF. This solution was heated to 50 °C for 1 hour, cooled to room  
13 temperature, and the reaction quenched by the addition of saturated aqueous  
14 NH<sub>4</sub>Cl. The solution was extracted with EtOAc and the combined organic  
15 layers were washed with H<sub>2</sub>O and saturated aqueous NaCl before being dried  
16 (MgSO<sub>4</sub>) and concentrated under reduced pressure. The title compound,  
17 177.0 mg (79%), was isolated by column chromatography (5% EtOAc /  
18 hexanes) as a pale-yellow solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 7.88 (1H, d, J  
19 = 7.8 Hz), 7.28-7.18 (9H, m), 5.84 (1H, s), 4.39 (2H, q, J = 7.1 Hz), 2.42  
20 (3H, s), 1.48 (6H, s), 1.40 (3H, t, J = 7.1 Hz).

21 Ethyl 4-[[4-(4-ethylphenyl)-2,2-dimethyl-(2H)-thiochromen-6-yl]-ethynyl]-2-  
22 fluorobenzoate (Compound 237)

23 A solution of 4-ethylbromobenzene (185.0 mg, 1.00 mmol) in 2.0 mL  
24 of THF was cooled to -78 °C and *tert*-butyllithium (128.5 mg, 2.0 mmol, 1.2  
25 mL of a 1.7M solution in pentane) was added to give a yellow solution.  
26 After 30 minutes a solution of ZnCl<sub>2</sub> (204.5 mg, 1.5 mmol) in 4.0 mL THF  
27 was slowly added via cannula. The resulting solution was warmed to room  
28 temperature and transferred via cannula to a solution of ethyl 4-(2,2-  
29 dimethyl-4-trifluoromethanesulfonyloxy-(2H)-thiochromen-6-ylethynyl)-2-

1 fluorobenzoate (Compound 234, 200.0 mg, 0.39 mmol) and  
2 tetrakis(triphenylphosphine)palladium(0) (24.0 mg, 0.02 mmol) in 2.0 mL  
3 THF. This solution was heated to 50 °C for 1 hour, cooled to room  
4 temperature, and the reaction quenched by the addition of saturated aqueous  
5  $\text{NH}_4\text{Cl}$ . The solution was extracted with EtOAc and the combined organic  
6 layers were washed with  $\text{H}_2\text{O}$  and saturated aqueous NaCl before being dried  
7 ( $\text{MgSO}_4$ ) and concentrated under reduced pressure. The title compound,  
8 158.0 mg (86%), was isolated by column chromatography (5% EtOAc /  
9 hexanes) as a pale-yellow solid.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ : 7.88 (1H, d, J  
10 = 7.8 Hz), 7.39-7.20 (9H, m), 5.86 (1H, s), 4.40 (2H, q, J = 7.1 Hz), 2.72 (2H,  
11 q, J = 7.6 Hz), 1.49 (6H, s), 1.40 (3H, t, J = 7.1 Hz), 1.30 (3H, t, J = 7.6  
12 Hz).

13 Ethyl 6-[(2,2-dimethyl-4-oxo-thiochroman-6-yl)ethynyl]-nicotinate (Compound  
14 238)

15 A solution of 6-ethynyl-2,2-dimethylthiochroman-4-one (Compound  
16 222, 510.0 mg, 2.36 mmol) and ethyl 6-iodonicotinate (654.0 mg, 2.36 mmol)  
17 in 20.0 mL  $\text{Et}_3\text{N}$  was purged with argon for 20 minutes. To this solution was  
18 added bis(triphenylphosphine)-palladium(II) chloride (425.0 mg, 0.60 mmol)  
19 and copper(I) iodide (115.0 mg, 0.60 mmol). After sparging for an additional  
20 10 minutes with argon, the solution was stirred overnight at room  
21 temperature. The reaction mixture was diluted with EtOAc (20 mL) and  
22 filtered through a pad of Celite using an  $\text{Et}_2\text{O}$  wash. Concentration of the  
23 filtrate under reduced pressure, followed by column chromatography (10 to  
24 20% EtOAc / hexanes) of the residual solid afforded 675 mg (78%) of the  
25 title compound as an orange solid.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ : 9.20 (1H,  
26 d, J = 1.0 Hz), 8.34 (1H, d, J = 1.8 Hz), 8.29 (1H, dd, J = 2.2, 8.2 Hz), 7.60  
27 (2H, m), 7.25 (1H, d, J = 8.4 Hz), 4.43 (2H, q, J = 7.1 Hz), 2.90 (2H, s), 1.49  
28 (6H, s), 1.43 (3H, t, J = 7.1 Hz).

29 Ethyl 6-(2,2-dimethyl-4-trifluoromethanesulfonyloxy-(2H)-thiochromen-6-

1 ylethynyl)-nicotinate (Compound 239)

2 A solution of sodium bis(trimethylsilyl)amide (284.2 mg, 1.55 mmol) in  
3 3.6 mL of THF was cooled to -78 °C and a solution of ethyl 6-(2,2-dimethyl-  
4 4-oxo-thiochroman-6-ylethynyl)-nicotinate (Compound 238, 480.0 mg, 1.31  
5 mmol) in 3.0 mL THF was added slowly. After 30 minutes a solution of 2-  
6 [N,N-bis(trifluoromethanesulfonyl)amino]-5-pyridine (608.0 mg, 1.55 mmol)  
7 in 3.0 mL of THF was added. After 5 minutes the solution was warmed to  
8 room temperature and stirred overnight. The reaction was quenched by the  
9 addition of saturated aqueous NH<sub>4</sub>Cl and extracted with EtOAc. The  
10 combined organic layers were washed with 5% aqueous NaOH and H<sub>2</sub>O  
11 before being dried (MgSO<sub>4</sub>) and concentrated under reduced pressure. The  
12 title compound was isolated by column chromatography (20% EtOAc /  
13 hexanes) as a yellow solid, 566.0 mg (87%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ:  
14 9.21 (1H, d, J = 2.0 Hz), 8.30 (1H, dd, J = 2.0, 8.1 Hz), 7.68 (1H, d, J = 1.5  
15 Hz), 7.61 (1H, d, J = 8.2 Hz), 7.49 (1H, dd, J = 1.7, 8.1 Hz), 7.31 (1H, d, J  
16 = 8.2 Hz), 5.92 (1H, s), 4.41 (2H, q, J = 7.1 Hz), 1.53 (6H, s), 1.43 (3H, t, J  
17 = 7.1 Hz).

18 Ethyl 6-[[4-(4-methylphenyl)-2,2-dimethyl-(2H)-thiochromen-6-yl]-ethynyl]-  
19 nicotinate (Compound 240)

20 A solution of 4-methylbromobenzene (280.0 mg, 1.64 mmol) in 2.0 mL  
21 of THF was cooled to -78 °C and *tert*-butyllithium (209.5 mg, 3.27 mmol, 1.9  
22 ml of a 1.7M solution in pentane) was added to give a yellow solution. After  
23 30 minutes a solution of ZnCl<sub>2</sub> (680.0 mg, 5.0 mmol) in 4.0 mL THF was  
24 slowly added via cannula. The resulting solution was warmed to room  
25 temperature and transferred via cannula to a solution of ethyl 6-(2,2-  
26 dimethyl-4-trifluoromethanesulfonyloxy-(2H)-thiochromen-6-ylethynyl)-  
27 nicotinate (Compound 239, 120.0 mg, 0.24 mmol) and  
28 tetrakis(triphenylphosphine)palladium(0) (20.0 mg, 0.02 mmol) in 2.0 mL  
29 THF. This solution was heated to 50 °C for 2 hours, cooled to room



1 temperature, and the reaction quenched by the addition of saturated aqueous  
2  $\text{NH}_4\text{Cl}$ . The solution was extracted with EtOAc and the combined organic  
3 layers were washed with  $\text{H}_2\text{O}$  and saturated aqueous NaCl before being dried  
4 ( $\text{MgSO}_4$ ) and concentrated under reduced pressure. The title compound,  
5 80.0 mg (76%), was isolated by column chromatography (10 to 15% EtOAc /  
6 hexanes) as a colorless solid.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ : 9.15 (1H, d,  $J$  =  
7 2.2 Hz), 8.23 (1H, dd,  $J$  = 2.1, 8.0 Hz), 7.51 (1H, d,  $J$  = 8.1 Hz), 7.40-7.32  
8 (3H, m), 7.18 (4H, m), 5.83 (1H, s), 4.40 (2H, q,  $J$  = 7.1 Hz), 2.40 (3H, s),  
9 1.47 (6H, s), 1.40 (3H, t,  $J$  = 7.1 Hz).

10 Ethyl 6-[[4-(4-ethylphenyl)-2,2-dimethyl-(2H)-thiochromen-6-yl]-ethynyl]-  
11 nicotinate (Compound 241)

12 A solution of 4-ethylbromobenzene (300.0 mg, 1.62 mmol) in 2.0 mL  
13 of THF was cooled to  $-78^\circ\text{C}$  and *tert*-butyllithium (207.6 mg, 3.24 mmol, 1.9  
14 mL of a 1.7M solution in pentane) was added to give a yellow solution.  
15 After 30 minutes a solution of  $\text{ZnCl}_2$  (408.0 mg, 3.0 mmol) in 4.0 mL THF  
16 was slowly added via cannula. The resulting solution was warmed to room  
17 temperature and transferred via cannula to a solution of ethyl 6-(2,2-  
18 dimethyl-4-trifluoromethanesulfonyloxy-(2H)-thiochromen-6-ylethynyl)-  
19 nicotinate (Compound 239, 178.0 mg, 0.36 mmol) and  
20 tetrakis(triphenylphosphine)palladium(0) (24 mg, 0.02 mmol) in 2.0 mL THF.  
21 This solution was heated to  $50^\circ\text{C}$  for 1 hour, cooled to room temperature,  
22 and the reaction quenched by the addition of saturated aqueous  $\text{NH}_4\text{Cl}$ . This  
23 solution was extracted with EtOAc and the combined organic layers were  
24 washed with  $\text{H}_2\text{O}$  and saturated aqueous NaCl before being dried ( $\text{MgSO}_4$ )  
25 and concentrated under reduced pressure. The title compound was isolated  
26 by column chromatography (10 to 15% EtOAc / hexanes) and recrystallization  
27 from  $\text{CH}_3\text{CN}$  to give 136.0 mg (83%) of pale yellow crystals.  $^1\text{H}$  NMR (300  
28 MHz,  $\text{CDCl}_3$ )  $\delta$ : 9.15 (1H, s), 8.23 (1H, dd,  $J$  = 2.2, 8.2 Hz), 7.51 (1H, d,  $J$  =  
29 8.1 Hz), 7.49-7.34 (3H, m), 7.24-7.17 (4H, m), 5.83 (1H, s), 4.40 (2H, q,  $J$  =

1 7.1 Hz), 2.70 (2H, q, J = 7.6 Hz), 1.47 (6H, s), 1.40 (3H, t, J = 7.1 Hz), 1.29  
2 (3H, t, J = 7.1 Hz).

3 4-[[4-phenyl-2,2-dimethyl-(2H)-thiochromen-6-yl]-ethynyl]-benzoic acid  
4 (Compound 242)

5 To a solution of ethyl 4-[[4-phenyl-2,2-dimethyl-(2H)-thiochromen-6-  
6 yl]-ethynyl]-benzoate (Compound 225, 105.0 mg, 0.247 mmol) in 3.0 mL THF  
7 and 3.0 mL EtOH was added NaOH (120.0 mg, 3.0 mmol, 3.0 mL of a 1M  
8 aqueous solution). The resulting solution was heated to 45 °C and stirred  
9 overnight. Upon cooling to room temperature the reaction mixture was  
10 acidified with 10% aqueous HCl and extracted with EtOAc. The combined  
11 organic layers were washed with H<sub>2</sub>O, saturated aqueous NaCl, and dried  
12 (Na<sub>2</sub>SO<sub>4</sub>) before removing the solvent under reduced pressure. The residual  
13 solid was recrystallized from CH<sub>3</sub>CN to give the title compound as a pale-  
14 yellow solid. <sup>1</sup>H NMR (300 MHz, d<sub>6</sub>-acetone) δ: 8.00 (2H, d, J = 8.4 Hz),  
15 7.57 (2H, d, J = 8.4 Hz), 7.48-7.29 (7H, m), 7.18 (1H, d, J = 1.3 Hz), 5.96  
16 (1H, s), 1.48 (6H, s).

17 4-[[4-(4-methylphenyl)-2,2-dimethyl-(2H)-thiochromen-6-yl]-ethynyl]-benzoic  
18 acid (Compound 243)

19 To a solution of ethyl 4-[[4-(4-methylphenyl)-2,2-dimethyl-(2H)-  
20 thiochromen-6-yl]-ethynyl]-benzoate (Compound 226, 126.0 mg, 0.287 mmol)  
21 in 3.0 mL THF and 3.0 mL EtOH was added NaOH (160.0 mg, 4.0 mmol,  
22 2.0 mL of a 2M aqueous solution). The resulting solution was heated to 45  
23 °C and stirred overnight. Upon cooling to room temperature the reaction  
24 mixture was acidified with 10% aqueous HCl and extracted with EtOAc. The  
25 combined organic layers were washed with H<sub>2</sub>O, saturated aqueous NaCl, and  
26 dried (Na<sub>2</sub>SO<sub>4</sub>) before removing the solvent under reduced pressure. The  
27 residual solid was recrystallized from CH<sub>3</sub>CN to give 85.0 mg (72%) of the  
28 title compound as a pale-yellow solid. <sup>1</sup>H NMR (300 MHz, d<sub>6</sub>-acetone) δ:  
29 8.00 (2H, d, J = 8.3 Hz), 7.58 (2H, d, J = 8.3 Hz), 7.45-7.38 (2H, m), 7.28-

1 7.17 (5H, m), 5.92 (1H, s), 2.37 (3H, s), 1.47 (6H, s).

2 4-[[4-(4-ethylphenyl)-2,2-dimethyl-(2H)-thiochromen-6-yl]-ethynyl]-benzoic  
3 acid (Compound 244)

4 To a solution of ethyl 4-[[4-(4-ethylphenyl)-2,2-dimethyl-(2H)-  
5 thiochromen-6-yl]-ethynyl]-benzoate (Compound 227, 940.0 mg, 2.08 mmol)  
6 in 10.0 mL THF and 5.0 mL EtOH was added NaOH (416.0 mg, 10.4 mmol,  
7 5.2 mL of a 2M aqueous solution). The resulting solution was stirred  
8 overnight at room temperature. The reaction mixture was acidified with 10%  
9 aqueous HCl and extracted with EtOAc. The combined organic layers were  
10 washed with H<sub>2</sub>O, saturated aqueous NaCl, and dried (Na<sub>2</sub>SO<sub>4</sub>) before  
11 removing the solvent under reduced pressure. The residual solid was  
12 recrystallized from CH<sub>3</sub>CN to give 786.0 mg (89%) of the title compound as  
13 a colorless solid. <sup>1</sup>H NMR (300 MHz, d<sub>6</sub>-acetone) δ: 8.01 (2H, d, J = 8.3 Hz),  
14 7.60 (2H, d, J = 8.5 Hz), 7.42 (2H, m), 7.29 (2H, m), 7.22 (3H, m), 5.94 (1H,  
15 s), 2.69 (2H, q, J = 7.7 Hz), 1.47 (6H, s), 1.25 (3H, t, J = 7.7 Hz).

16 4-[[4-(4-isopropylphenyl)-2,2-dimethyl-(2H)-thiochromen-6-yl]-ethynyl]-  
17 benzoic acid (Compound 245)

18 To a solution of ethyl 4-[[4-(4-isopropylphenyl)-2,2-dimethyl-(2H)-  
19 thiochromen-6-yl]-ethynyl]-benzoate (Compound 228, 89.0 mg, 0.19 mmol) in  
20 3.0 mL THF and 3.0 mL EtOH was added NaOH (80.0 mg, 2.0 mmol, 2.0  
21 mL of a 1M aqueous solution). The resulting solution was heated to 45 °C  
22 and stirred overnight. Upon cooling to room temperature the reaction  
23 mixture was acidified with 10% aqueous HCl and extracted with EtOAc. The  
24 combined organic layers were washed with H<sub>2</sub>O, saturated aqueous NaCl, and  
25 dried (Na<sub>2</sub>SO<sub>4</sub>) before removing the solvent under reduced pressure. The  
26 residual solid was recrystallized from CH<sub>3</sub>CN to give 78.0 mg (93%) of the  
27 title compound as a pale-yellow solid. <sup>1</sup>H NMR (300 MHz, d<sub>6</sub>-acetone) δ:  
28 8.00 (2H, d, J = 8.4 Hz), 7.58 (2H, d, J = 8.4 Hz), 7.45-7.21 (7H, m), 5.93  
29 (1H, s), 2.95 (1H, hept, J = 7.0 Hz), 1.47 (6H, s), 1.25 (6H, d, J = 7.0 Hz).

1 4-[[4-(4-*tert*-butylphenyl)-2,2-dimethyl-(2H)-thiochromen-6-yl]-ethynyl]-benzoic  
2 acid (Compound 246)

3 To a solution of ethyl 4-[[4-(4-*tert*-butylphenyl)-2,2-dimethyl-(2H)-  
4 thiochromen-6-yl]-ethynyl]-benzoate (Compound 229, 65.0 mg, 0.135 mmol)  
5 in 2.0 mL THF and 2.0 mL EtOH was added NaOH (80.0 mg, 2.0 mmol, 2.0  
6 mL of a 1M aqueous solution). The resulting solution was heated to 45 °C  
7 and stirred overnight. Upon cooling to room temperature the reaction  
8 mixture was acidified with 10% aqueous HCl and extracted with EtOAc. The  
9 combined organic layers were washed with H<sub>2</sub>O, saturated aqueous NaCl, and  
10 dried (Na<sub>2</sub>SO<sub>4</sub>) before removing the solvent under reduced pressure. The  
11 residual solid was recrystallized from CH<sub>3</sub>CN to give 33.0 mg (54%) of the  
12 title compound as a pale-yellow solid. <sup>1</sup>H NMR (300 MHz, d<sub>6</sub>-acetone) δ:  
13 8.01 (2H, d, J = 8.4 Hz), 7.57 (2H, d, J = 8.4 Hz), 7.45 (4H, m), 7.24 (3H,  
14 m), 5.93 (1H, s), 1.47 (6H, s), 1.34 (9H, s).

15 4-[[4-(5-methyl-thiophen-2-yl)-2,2-dimethyl-(2H)-thiochromen-6-yl]-ethynyl]-  
16 benzoic acid (Compound 247)

17 To a solution of ethyl 4-[[4-(5-methylthiophen-2-yl)-2,2-dimethyl-(2H)-  
18 thiochromen-6-yl]-ethynyl]-benzoate (Compound 230, 143.0 mg, 0.322 mmol)  
19 in 3.0 mL THF and 3.0 mL EtOH was added NaOH (160.0 mg, 4.0 mmol,  
20 4.0 mL of a 1M aqueous solution). The resulting solution was stirred  
21 overnight at room temperature. The reaction mixture was acidified with 10%  
22 aqueous HCl and extracted with EtOAc. The combined organic layers were  
23 washed with H<sub>2</sub>O, saturated aqueous NaCl, and dried (Na<sub>2</sub>SO<sub>4</sub>) before  
24 removing the solvent under reduced pressure. The residual solid was  
25 recrystallized from CH<sub>3</sub>CN to give 110.0 mg (82%) of the title compound as  
26 a pale-yellow solid. <sup>1</sup>H NMR (300 MHz, d<sub>6</sub>-acetone) δ: 8.03 (2H, d, J = 8.4  
27 Hz), 7.63 (2H, d, J = 8.3 Hz), 7.60 (1H, s), 7.44 (2H, s), 6.87 (1H, d, J = 3.5  
28 Hz), 6.79 (1H, m), 6.10 (1H, s), 2.49 (3H, s), 1.45 (6H, s).

29 4-[[4-(5-ethyl-thiophen-2-yl)-2,2-dimethyl-(2H)-thiochromen-6-yl]-ethynyl]-

1 benzoic acid (Compound 248)

2 To a solution of ethyl 4-[[4-(5-ethylthiophen-2-yl)-2,2-dimethyl-(2H)-  
3 thiochromen-6-yl]-ethynyl]-benzoate (Compound 231, 23.0 mg, 0.05 mmol) in  
4 1.0 mL THF and 1.0 mL EtOH was added NaOH (40.0 mg, 1.0 mmol, 1.0  
5 mL of a 1M aqueous solution). The resulting solution was stirred overnight  
6 at room temperature. The reaction mixture was acidified with 10% aqueous  
7 HCl and extracted with EtOAc. The combined organic layers were washed  
8 with H<sub>2</sub>O, saturated aqueous NaCl, and dried (Na<sub>2</sub>SO<sub>4</sub>) before removing the  
9 solvent under reduced pressure. The residual solid was recrystallized from  
10 CH<sub>3</sub>CN to give 15.5 mg (72%) of the title compound as an orange solid. <sup>1</sup>H  
11 NMR (300 MHz, d<sub>6</sub>-acetone) δ: 8.03 (2H, d, J = 8.3 Hz), 7.63 (2H, d, J =  
12 8.3 Hz), 7.61 (1H, s), 7.44 (2H, s), 6.90 (1H, d, J = 3.5 Hz), 6.83 (1H, d, J =  
13 3.5 Hz), 6.10 (1H, s), 2.86 (2H, q, J = 7.6 Hz), 1.45 (6H, s), 1.30 (3H, t, J =  
14 7.6 Hz).

15 4-[[4-(5-*tert*-butylthiophen-2-yl)-2,2-dimethyl-(2H)-thiochromen-6-yl]-ethynyl]-  
16 benzoic acid (Compound 249)

17 To a solution of ethyl 4-[[4-(5-*tert*-butylthiophen-2-yl)-2,2-dimethyl-  
18 (2H)-thiochromen-6-yl]-ethynyl]-benzoate (Compound 232, 88.0 mg, 0.181  
19 mmol) in 2.0 mL THF and 2.0 mL EtOH was added NaOH (160.0 mg, 4.0  
20 mmol, 4.0 mL of a 1M aqueous solution). The resulting solution was stirred  
21 overnight at room temperature. The reaction mixture was acidified with  
22 10% aqueous HCl and extracted with EtOAc. The combined organic layers  
23 were washed with H<sub>2</sub>O, saturated aqueous NaCl, and dried (Na<sub>2</sub>SO<sub>4</sub>) before  
24 removing the solvent under reduced pressure. The residual solid was  
25 recrystallized from CH<sub>3</sub>CN to give 68.0 mg (82%) of the title compound as a  
26 pale-yellow solid. <sup>1</sup>H NMR (300 MHz, d<sub>6</sub>-acetone) δ: 8.03 (2H, d, J = 8.3  
27 Hz), 7.63 (2H, d, J = 8.3 Hz), 7.62 (1H, s), 7.44 (2H, d, J = 1.2 Hz), 6.87  
28 (2H, s), 6.11 (1H, s), 1.45 (6H, s), 1.39 (9H, s).

29 4-[[4-(6-methylpyridin-3-yl)-2,2-dimethyl-(2H)-thiochromen-6-yl]-ethynyl]-

1 benzoic acid (Compound 250)

2 To a solution of ethyl 4-[[4-(6-methylpyridin-3-yl)-2,2-dimethyl-(2H)-  
3 thiochromen-6-yl]-ethynyl]-benzoate (Compound 233, 70.0 mg, 0.159 mmol)  
4 in 3.0 mL THF and 3.0 mL EtOH was added NaOH (120.0 mg, 3.0 mmol,  
5 3.0 mL of a 1M aqueous solution). The resulting solution was stirred  
6 overnight at 35 °C. Upon cooling to room temperature, the reaction mixture  
7 was acidified with 10% aqueous HCl and extracted with EtOAc. The  
8 combined organic layers were washed with H<sub>2</sub>O, saturated aqueous NaCl, and  
9 dried (Na<sub>2</sub>SO<sub>4</sub>) before removing the solvent under reduced pressure. The  
10 residual solid was recrystallized from acetone to give 60.0 mg (92%) of the  
11 title compound as a colorless solid. <sup>1</sup>H NMR (300 MHz, d<sub>6</sub>-acetone) δ: 8.36  
12 (1H, d, J = 2.1 Hz), 7.91 (2H, d, J = 8.3 Hz), 7.60 (2H, d, J = 8.3 Hz), 7.56  
13 (1H, dd, J = 2.1 8.0 Hz), 7.45 (2H, m), 7.31 (1H, d, J = 8.0 Hz), 7.06 (1H,  
14 s), 6.06 (1H, s), 2.51 (3H, s), 1.44 (6H, s).

15 4-[[4-(4-methylphenyl)-2,2-dimethyl-(2H)-thiochromen-6-yl]-ethynyl]-2-  
16 fluorobenzoic acid (Compound 251)

17 To a solution of ethyl 4-[[4-(4-methylphenyl)-2,2-dimethyl-(2H)-  
18 thiochromen-6-yl]-ethynyl]-2-fluorobenzoate (Compound 236, 100.0 mg, 0.219  
19 mmol) in 3.0 mL THF and 3.0 mL EtOH was added NaOH (80.0 mg, 2.0  
20 mmol, 2.0 mL of a 1M aqueous solution). The resulting solution was heated  
21 to 40 °C and stirred overnight. Upon cooling to room temperature the  
22 reaction mixture was acidified with 10% aqueous HCl and extracted with  
23 EtOAc. The combined organic layers were washed with H<sub>2</sub>O, saturated  
24 aqueous NaCl, and dried (Na<sub>2</sub>SO<sub>4</sub>) before removing the solvent under  
25 reduced pressure. The residual solid was recrystallized from CH<sub>3</sub>CN to  
26 give 78.0 mg (83%) of the title compound as a pale-yellow solid. <sup>1</sup>H NMR  
27 (300 MHz, d<sub>6</sub>-acetone) δ: 7.94 (1H, d, J = 7.8 Hz), 7.43-7.17 (9H, m), 5.93  
28 (1H, s), 2.38 (3H, s), 1.47 (6H, s).

29 4-[[4-(4-ethylphenyl)-2,2-dimethyl-(2H)-thiochromen-6-yl]-ethynyl]-2-

1 fluorobenzoic acid (Compound 252)

2 To a solution of ethyl 4-[[4-(4-ethylphenyl)-2,2-dimethyl-(2H)-  
3 thiochromen-6-yl]-ethynyl]-2-fluorobenzoate (Compound 237, 125.0 mg, 0.266  
4 mmol) in 3.0 mL THF and 3.0 mL EtOH was added NaOH (120.0 mg, 3.0  
5 mmol, 3.0 mL of a 1M aqueous solution). The resulting solution was stirred  
6 overnight at room temperature. The reaction mixture was acidified with 10%  
7 aqueous HCl and extracted with EtOAc. The combined organic layers were  
8 washed with H<sub>2</sub>O, saturated aqueous NaCl, and dried (Na<sub>2</sub>SO<sub>4</sub>) before  
9 removing the solvent under reduced pressure. The residual solid was  
10 recrystallized from CH<sub>3</sub>CN to give 100.0 mg (86%) of the title compound as  
11 a pale-yellow solid. <sup>1</sup>H NMR (300 MHz, d<sub>6</sub>-acetone) δ: 7.94 (1H, d, J = 7.8  
12 Hz), 7.43-7.20 (9H, m), 5.93 (1H, s), 2.68 (2H, q, J = 7.6 Hz), 1.47 (6H, s),  
13 1.24 (3H, t, J = 7.6 Hz).

14 6-[[4-(4-methylphenyl)-2,2-dimethyl-(2H)-thiochromen-6-yl]-ethynyl]-nicotinic  
15 acid (Compound 253)

16 To a solution of ethyl 6-[[4-(4-methylphenyl)-2,2-dimethyl-(2H)-  
17 thiochromen-6-yl]-ethynyl]-nicotinate (Compound 240, 66.0 mg, 0.15 mmol)  
18 in 2.0 mL THF and 2.0 mL EtOH was added NaOH (80.0 mg, 2.0 mmol, 2.0  
19 mL of a 1M aqueous solution). The resulting solution was heated to 45 °C  
20 and stirred overnight. Upon cooling to room temperature the reaction  
21 mixture was acidified with 10% aqueous HCl and extracted with EtOAc. The  
22 combined organic layers were washed with H<sub>2</sub>O, saturated aqueous NaCl, and  
23 dried (Na<sub>2</sub>SO<sub>4</sub>) before removing the solvent under reduced pressure to give  
24 the title compound as a yellow solid, 50.0 mg (81%). <sup>1</sup>H NMR (300 MHz,  
25 d<sub>6</sub>-acetone) δ: 9.09 (1H, d, J = 1.4 Hz), 8.30 (1H, dd, J = 2.0, 8.1 Hz), 7.65  
26 (1H, d, J = 8.1 Hz), 7.46 (2H, s), 7.24 (5H, m), 5.94 (1H, s), 2.37 (3H, s),  
27 1.48 (6H, s).

28 6-[[4-(4-ethylphenyl)-2,2-dimethyl-(2H)-thiochromen-6-yl]-ethynyl]-nicotinic  
29 acid (Compound 254)

1 To a solution of ethyl 6-[[4-(4-ethylphenyl)-2,2-dimethyl-(2H)-  
2 thiochromen-6-yl]-ethynyl]-nicotinate (Compound 241, 110.0 mg, 0.24 mmol)  
3 in 3.0 mL THF and 3.0 mL EtOH was added NaOH (120.0 mg, 3.0 mmol,  
4 3.0 mL of a 1M aqueous solution). This mixture was heated to 40 °C for 1  
5 hour, cooled to room temperature, and the resulting solution was stirred  
6 overnight. The reaction mixture was acidified with 10% aqueous HCl and  
7 extracted with EtOAc. The combined organic layers were washed with H<sub>2</sub>O,  
8 saturated aqueous NaCl, and dried (Na<sub>2</sub>SO<sub>4</sub>) before removing the solvent  
9 under reduced pressure to give 100 mg (96%) of the title compound as a  
10 colorless solid. <sup>1</sup>H NMR (300 MHz, d<sub>6</sub>-acetone) δ: 9.08 (1H, d, J = 2.0 Hz),  
11 8.30 (1H, dd, J = 2.2, 8.2 Hz), 7.66 (1H, d, J = 8.2 Hz), 7.46 (2H, s), 7.31-  
12 7.21 (5H, m), 5.95 (1H, s), 2.69 (2H, q, J = 7.6 Hz), 1.48 (6H, s), 1.25 (3H, t,  
13 J = 7.6 Hz).

14 4-[[4-(4-methylphenyl)-2,2-dimethyl-7-fluoro-(2H)-thiochromen-6-yl]-ethynyl]-  
15 benzoic acid (Compound 255)

16 To a solution of ethyl 4-[[4-(4-methylphenyl)-2,2-dimethyl-7-fluoro-  
17 (2H)-thiochromen-6-yl]-ethynyl]-benzoate (Compound 213, 108.0 mg, 0.236  
18 mmol) in 2.0 mL THF and 2.0 mL EtOH was added NaOH (120.0 mg, 3.0  
19 mmol, 3.0 mL of a 1M aqueous solution). The resulting solution was heated  
20 to 45 °C and stirred overnight. Upon cooling to room temperature the  
21 reaction mixture was acidified with 10% aqueous HCl and extracted with  
22 EtOAc. The combined organic layers were washed with H<sub>2</sub>O, saturated  
23 aqueous NaCl, and dried (Na<sub>2</sub>SO<sub>4</sub>) before removing the solvent under  
24 reduced pressure. The residual solid was recrystallized from CH<sub>3</sub>CN to give  
25 85 mg (80%) of the title compound as a pale-yellow solid. <sup>1</sup>H NMR (300  
26 MHz, d<sub>6</sub>-acetone) δ: 8.03 (2H, d, J = 8.3 Hz), 7.61 (2H, d, J = 8.3 Hz), 7.31-  
27 7.18 (6H, m), 5.90 (1H, s), 2.37 (3H, s), 1.48 (6H, s).

28 4-[[4-(4-methylphenyl)-2,2-dimethyl-7-methoxy-(2H)-thiochromen-6-yl]-  
29 ethynyl]-benzoic acid (Compound 256)



1 To a solution of ethyl 4-[[4-(4-methylphenyl)-2,2-dimethyl-7-methoxy-  
2 (2H)-thiochromen-6-yl]-ethynyl]-benzoate (Compound 215, 64.0 mg, 0.113  
3 mmol) in 2.0 mL THF and 2.0 mL EtOH was added NaOH (80.0 mg, 2.0  
4 mmol, 2.0 mL of a 1M aqueous solution). The resulting solution was heated  
5 to 45 °C and stirred overnight. Upon cooling to room temperature the  
6 reaction mixture was acidified with 10% aqueous HCl and extracted with  
7 EtOAc. The combined organic layers were washed with H<sub>2</sub>O, saturated  
8 aqueous NaCl, and dried (Na<sub>2</sub>SO<sub>4</sub>) before removing the solvent under  
9 reduced pressure. The residual solid was recrystallized from CH<sub>3</sub>CN to give  
10 50 mg (83%) of the title compound as a colorless solid. <sup>1</sup>H NMR (300 MHz,  
11 d<sub>6</sub>-acetone) δ: 8.00 (2H, d, J = 8.2 Hz), 7.56 (2H, d, J = 8.2 Hz), 7.28-7.14  
12 (5H, m), 7.06 (1H, s), 5.76 (1H, s), 3.96 (3H, s), 2.36 (3H, s), 1.47 (6H, s).  
13 4-[[4-(5-methyl-2-thienyl)-2,2-dimethyl-7-fluoro-(2H)-thiochromen-6-yl]-  
14 ethynyl]-benzoic acid (Compound 257)

15 To a solution of ethyl 4-[[4-(5-methyl-2-thienyl)-2,2-dimethyl-7-fluoro-  
16 (2H)-thiochromen-6-yl]-ethynyl]-benzoate (Compound 214, 58.0 mg, 0.125  
17 mmol) in 2.0 mL THF and 2.0 mL EtOH was added NaOH (80.0 mg, 2.0  
18 mmol, 2.0 mL of a 1M aqueous solution). The resulting solution was heated  
19 to 45 °C and stirred overnight. Upon cooling to room temperature the  
20 reaction mixture was acidified with 10% aqueous HCl and extracted with  
21 EtOAc. The combined organic layers were washed with H<sub>2</sub>O, saturated  
22 aqueous NaCl, and dried (Na<sub>2</sub>SO<sub>4</sub>) before removing the solvent under  
23 reduced pressure. The residual solid was recrystallized from CH<sub>3</sub>CN to give  
24 50 mg (90%) of the title compound as a pale-yellow solid. <sup>1</sup>H NMR (300  
25 MHz, d<sub>6</sub>-acetone) δ: 8.05 (2H, d, J = 8.3 Hz), 7.56 (2H, d, J = 8.3 Hz), 7.61  
26 (1H, d, J = 7.4 Hz), 7.31 (1H, d, J = 9.6 Hz), 6.89 (1H, d, J = 3.5 Hz), 6.79  
27 (1H, d, J = 3.5 Hz), 6.06 (1H, s), 2.49 (3H, s), 1.46 (6H, s).  
28 4-Bromophenylacetate (Compound 258)

29 To a solution of 4-bromophenol (20.0 g, 0.121 mol) in 200 mL CH<sub>2</sub>Cl<sub>2</sub>

1 was added triethylamine (14.0 g, 0.139 mol) followed by acetyl chloride (9.53  
2 g, 0.121 mol) in small portions at room temperature. After stirring for 21  
3 hours the mixture was poured into 200 mL H<sub>2</sub>O. The organic layer was  
4 washed with 5% aqueous NaOH, H<sub>2</sub>O, and dried over MgSO<sub>4</sub>. Removal of the  
5 solvent under reduced pressure and distillation of the residue afforded 20.6 g  
6 (83%) of the product as a colorless oil, bp 93-95 °C / 2 mm.

7 2-Hydroxy-5-bromo-acetophenone (Compound 259)

8 A mixture of AlCl<sub>3</sub> (6.20 g, 46.50 mmol) and 4-bromophenylacetate  
9 (Compound 258, 10.0 g, 46.50 mmol) was heated to 150 °C for 30 minutes  
10 while a stream of argon was passed over the molten solid. Upon cooling to  
11 room temperature the orange solid was carefully treated with 100 mL of H<sub>2</sub>O  
12 and 100 mL of 10% aqueous HCl. The resulting mixture was extracted with  
13 EtOAc and the combined organic layers were washed with H<sub>2</sub>O and saturated  
14 aqueous NaCl before being dried over Na<sub>2</sub>SO<sub>4</sub>. Concentration of the solution  
15 under reduced pressure afforded a tan solid which after recrystallization from *i*-  
16 PrOH provided 7.18 g (72%) of the title compound as an off-white crystalline  
17 solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ : 12.17 (1H, s), 7.84 (1H, d, J = 2.6 Hz),  
18 7.55 (1H, dd, J = 2.5, 9.0 Hz), 6.90 (1H, d, J = 8.9 Hz), 2.64 (3H, s).

19 2,2-Dimethyl-6-bromo-chroman-4-one (Compound 260)

20 To a solution of piperidine (2.83 g, 33.25 mmol) in 45 mL benzene was  
21 added trifluoroacetic acid (344.6 mg, 3.02 mmol) as a solution in 4.0 mL of  
22 benzene. Acetone ( 8.78 g, 151.3 mmol) was added followed by 2-hydroxy-5-  
23 bromoacetophenone (Compound 259, 6.50 g, 30.23 mmol). The resulting  
24 solution was heated to reflux in a flask fitted with a Dean-Stark trap. After 24  
25 hours, an additional 2.5 equivalents of acetone and 0.1 equivalents of  
26 trifluoroacetic acid were added. After a total of 43 hours the reaction was  
27 cooled to room temperature and diluted with EtOAc. The solution was  
28 washed with 1M aqueous HCl, H<sub>2</sub>O, and saturated aqueous NaCl before being  
29 dried (MgSO<sub>4</sub>) and concentrated under reduced pressure. Distillation of the  
30 residual oil (bulb-to-bulb) afforded 4.80g (62%) of the title compound as a

1 clear yellow oil, bp 105-115 °C / 5 mm. The reaction conditions here are a  
2 moderate modification of the synthesis of the title compound described by  
3 *Buckle et al.* in *J. Med. Chem.* 1990 3028-3034. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ  
4 : 7.97 (1H, d, J = 2.6 Hz), 7.54 (1H, dd, J = 2.6, 8.7 Hz), 6.84 (1H, d, J = 8.8  
5 Hz), 2.72 (2H, s), 1.46 (6H, s).

6 2,2-Dimethyl-6-trimethylsilanylethynyl-chroman-4-one (Compound 261)

7 A solution of 2,2-dimethyl-6-bromo-chroman-4-one (Compound 260,  
8 4.30 g, 16.85 mmol) and copper (I) iodide (321 mg, 0.17 mmol) in 55.0 mL  
9 Et<sub>3</sub>N was sparged with argon for 30 minutes. To this solution were added  
10 trimethylsilylacetylene (4.66 g, 50.56 mmol) and bis(triphenylphosphine)-  
11 palladium(II) chloride (1.18g, 0.17 mmol). The mixture was heated to 70 °C  
12 for 26 hours, cooled to room temperature, and diluted with Et<sub>2</sub>O (100 mL).  
13 The resulting mixture was filtered through a pad of Celite using an Et<sub>2</sub>O wash  
14 and the filtrate washed with H<sub>2</sub>O, a solution of NH<sub>4</sub>OH/ saturated aqueous  
15 NH<sub>4</sub>Cl (9:1), H<sub>2</sub>O, 1M aqueous HCl, H<sub>2</sub>O and saturated aqueous NaCl before  
16 being dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated under reduced pressure. Column  
17 chromatography (5% EtOAc - hexanes) of the residual oil afforded 4.09 g  
18 (89%) of the product as a yellow waxy solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ :  
19 7.99 (1H, d, J = 2.1 Hz), 7.53 (1H, dd, J = 2.2, 8.6 Hz), 6.86 (1H, d, J = 8.6  
20 Hz), 2.72 (2H, s), 1.45 (6H, s), 0.24 (9H, s).

21 2,2-Dimethyl-6-ethynyl-chroman-4-one (Compound 262)

22 To a solution of 2,2-dimethyl-6-trimethylsilanylethynyl-chroman-4-one  
23 (Compound 261, 4.05 g, 14.87 mmol) in 60.0 mL of MeOH was added K<sub>2</sub>CO<sub>3</sub>,  
24 (410.9 mg, 2.97 mmol). The resulting mixture was stirred at room temperature  
25 for 24 hours, diluted with EtOAc (100mL) and washed with H<sub>2</sub>O and saturated  
26 aqueous NaCl, and dried over Na<sub>2</sub>SO<sub>4</sub>. Concentration of this solution under  
27 reduced pressure afforded 2.71 g (91%) of the product as a tan solid. <sup>1</sup>H NMR  
28 (300 MHz, CDCl<sub>3</sub>) δ : 8.01 (1H, d, J = 2.2 Hz), 7.56 (1H, dd, J = 2.2, 8.6 Hz),  
29 6.90 (1H, d, J = 8.6 Hz), 3.02 (1H, s), 2.74 (2H, s), 1.47 (6H, s).

30 Ethyl 4-[(2,2-dimethyl-4-oxo-chroman-6-yl)ethynyl]-benzoate (Compound 263)

1 A solution of 6-ethynyl-2,2-dimethylchroman-4-one (Compound 262,  
2 2.60 g, 13.0 mmol) and ethyl 4-iodobenzoate (3.6 g, 13.0 mmol) in 50.0 mL  
3 Et<sub>3</sub>N was purged with argon for 15 minutes. To this solution was added  
4 bis(triphenylphosphine)-palladium(II) chloride (1.82 g, 2.6 mmol) and  
5 copper(I) iodide (496 mg, 2.6 mmol). After sparging for an additional 10  
6 minutes with argon, the solution was stirred overnight at room temperature.  
7 The reaction mixture was filtered through a pad of Celite using an Et<sub>2</sub>O  
8 wash. Concentration of the filtrate under reduced pressure, followed by  
9 column chromatography (2-5% EtOAc-hexanes) of the residual solid afforded  
10 2.28 g (50%) the title compound as an orange solid. <sup>1</sup>H NMR (300 MHz,  
11 CDCl<sub>3</sub>) δ : 8.06 (1H, d, J = 2.2 Hz), 8.02 (2H, d, J = 8.5 Hz), 2.61 (1H, dd, J  
12 = 2.2, 8.6 Hz), 7.55 (2H, d, J = 8.5 Hz), 6.93 (1H, d, J = 8.6 Hz), 4.39 (2H,  
13 q, J = 7.1 Hz), 2.75 (3H, s), 1.48 (6H, s), 1.41 (t, J = 7.1 Hz).

14 Ethyl 4-(2,2-dimethyl-4-trifluoromethanesulfonyloxy-(2H)-chromen-6-  
15 ylethynyl)-benzoate (Compound 264)

16 A solution of sodium bis(trimethylsilyl)amide (1.56g, 8.5 mmol) in 18.0  
17 mL of THF was cooled to -78 °C and a solution of ethyl 4-((2,2-dimethyl-4-  
18 oxo-chroman-6-yl)ethynyl)-benzoate (Compound 263, 2.26 g, 6.49 mmol) in  
19 15.0 mL was added slowly. After 30 minutes a solution of 2-[N,N-  
20 bis(trifluoromethanesulfonyl)amino]-5-pyridine (3.10 g, 7.79 mmol) in 10 mL  
21 of THF was added slowly. After 5 minutes the solution was warmed to room  
22 temperature and stirred overnight. The reaction was quenched by the  
23 addition of saturated aqueous NH<sub>4</sub>Cl and extracted with EtOAc. The  
24 combined organic layers were washed with 5% aqueous NaOH and H<sub>2</sub>O  
25 before being dried (MgSO<sub>4</sub>) and concentrated under reduced pressure. The  
26 title compound, 2.0 g (64%), was isolated by column chromatography (5%  
27 EtOAc-hexanes) as a colorless solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 8.03  
28 (2H, d, J = 8.3 Hz), 7.57 (2H, d, J = 8.3 Hz), 7.45-7.21 (2H, m), 6.85 (1H, d,  
29 J = 8.4 Hz), 5.69 (1H, s), 4.40 (2H, q, J = 7.1 Hz), 1.55 (6H, s), 1.4 (3H, t, J

1 = 7.1 Hz).

2 Ethyl 4-[[4-phenyl-2,2-dimethyl-(2H)-chromen-6-yl]-ethynyl]-benzoate

3 (Compound 265)

4 A solution of bromobenzene (250.0 mg, 1.59 mmol) in 3.0 mL of THF  
5 was cooled to -78 °C and *tert*-butyllithium (203.7 mg, 3.18 mmol, 1.9 ml of a  
6 1.7M solution in pentane) was added to give a yellow solution. After 30  
7 minutes a solution of ZnCl<sub>2</sub> (440.0 mg, 3.18 mmol) in 5.0 mL THF was slowly  
8 added via cannula. The resulting solution was warmed to room temperature  
9 and transferred via cannula to a solution of ethyl 4-(2,2-dimethyl-4-  
10 trifluoromethanesulfonyloxy-(2H)-chromen-6-ylethynyl)-benzoate (Compound  
11 264, 150.0 mg, 0.31 mmol) and tetrakis(triphenylphosphine)palladium(0) (24.0  
12 mg, 0.02 mmol) in 2.0 mL THF. This solution was heated to 50 °C for 2  
13 hours, cooled to room temperature, and the reaction quenched by the  
14 addition of saturated aqueous NH<sub>4</sub>Cl. This solution was extracted with  
15 EtOAc and the combined organic layers were washed with H<sub>2</sub>O and saturated  
16 aqueous NaCl before being dried (MgSO<sub>4</sub>) and concentrated under reduced  
17 pressure. The title compound, 110.0 mg (87%), was isolated by column  
18 chromatography (2.5% EtOAc / hexanes) as a colorless solid. <sup>1</sup>H NMR (300  
19 MHz, CDCl<sub>3</sub>) δ: 7.99 (2H, d, J = 8.3 Hz), 7.51 (2H, d, J = 8.3 Hz), 7.47-7.35  
20 (6H, m), 7.21 (1H, d, J = 2.0 Hz), 6.88 (1H, d, J = 8.4 Hz), 5.66 (1H, s),  
21 4.38 (2H, q, J = 7.1 Hz), 1.52 (6H, s), 1.40 (3H, t, J = 7.1 Hz).

22 Ethyl 4-[[4-(4-methylphenyl)-2,2-dimethyl-(2H)-chromen-6-yl]-ethynyl]-

23 benzoate (Compound 266)

24 A solution of 4-methylbromobenzene (237.0 mg, 1.38 mmol) in 3.0 mL  
25 of THF was cooled to -78 °C and *tert*-butyllithium (177.5 mg, 2.77 mmol, 1.6  
26 ml of a 1.7M solution in pentane) was added to give a yellow solution. After  
27 30 minutes a solution of ZnCl<sub>2</sub> (377.0 mg, 2.77 mmol) in 5.0 mL THF was  
28 slowly added via cannula. The resulting solution was warmed to room  
29 temperature and transferred via cannula to a solution of ethyl 4-(2,2-

1 dimethyl-4-trifluoromethanesulfonyloxy-(2H)-chromen-6-ylethynyl)-benzoate  
2 (Compound 264, 150.0 mg, 0.31 mmol) and  
3 tetrakis(triphenylphosphine)palladium(0) (24.0 mg, 0.02 mmol) in 2.0 mL  
4 THF. This solution was heated to 50 °C for 2 hours, cooled to room  
5 temperature, and the reaction quenched by the addition of saturated aqueous  
6 NH<sub>4</sub>Cl. This solution was extracted with EtOAc and the combined organic  
7 layers were washed with H<sub>2</sub>O and saturated aqueous NaCl before being dried  
8 (MgSO<sub>4</sub>) and concentrated under reduced pressure. The title compound,  
9 120.0 mg (92%), was isolated by column chromatography (2.5% EtOAc /  
10 hexanes) as a colorless solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 7.98 (2H, d, J =  
11 8.5 Hz), 7.50 (2H, d, J = 8.5 Hz), 7.34 (1H, dd, J = 2.1, 8.3 Hz), 7.25-7.21  
12 (5H, m), 6.85 (1H, d, J = 8.3 Hz), 5.62 (1H, s), 4.36 (2H, q, J = 7.1 Hz), 2.41  
13 (3H, s), 1.49 (6H, s), 1.39 (3H, t, J = 7.1 Hz).

14 Ethyl 4-[[4-(4-ethylphenyl)-2,2-dimethyl-(2H)-chromen-6-yl]-ethynyl]-benzoate  
15 (Compound 267)

16 A solution of 4-ethylbromobenzene (280.0 mg, 1.51 mmol) in 3.0 mL  
17 of THF was cooled to -78 °C and *tert*-butyllithium (198.6 mg, 3.10 mmol, 1.9  
18 ml of a 1.7M solution in pentane) was added to give a yellow solution. After  
19 30 minutes a solution of ZnCl<sub>2</sub> (408.0 mg, 3.10 mmol) in 5.0 mL THF was  
20 slowly added via cannula. The resulting solution was warmed to room  
21 temperature and transferred via cannula to a solution of ethyl 4-(2,2-  
22 dimethyl-4-trifluoromethanesulfonyloxy-(2H)-chromen-6-ylethynyl)-benzoate  
23 (Compound 264, 150.0 mg, 0.31 mmol) and  
24 tetrakis(triphenylphosphine)palladium(0) (24.0 mg, 0.02 mmol) in 2.0 mL  
25 THF. This solution was heated to 50 °C for 2 hours, cooled to room  
26 temperature, and the reaction quenched by the addition of saturated aqueous  
27 NH<sub>4</sub>Cl. This solution was extracted with EtOAc and the combined organic  
28 layers were washed with H<sub>2</sub>O and saturated aqueous NaCl before being dried  
29 (MgSO<sub>4</sub>) and concentrated under reduced pressure. The title compound,

1 123.0 mg (91%), was isolated by column chromatography (2.5% EtOAc /  
2 hexanes) as a pale-yellow solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 8.00 (2H, d, J  
3 = 8.4 Hz), 7.52 (2H, d, J = 8.4 Hz), 7.36 (1H, dd, J = 2.0, 8.3 Hz), 7.31-7.25  
4 (5H, m), 6.88 (1H, d, J = 8.3 Hz), 5.65 (1H, s), 4.38 (2H, q, J = 7.1 Hz), 2.73  
5 (2H, q, J = 7.6 Hz), 1.52 (6H, s), 1.41 (3H, t, J = 7.1 Hz), 1.31 (2H, t, J =  
6 7.6 Hz).

7 Ethyl 4-[[4-(4-(1-methylethyl)phenyl)-2,2-dimethyl-(2H)-chromen-6-yl]-  
8 ethynyl]-benzoate (Compound 268)

9 A solution of 4-*iso*-propylbromobenzene (250.0 mg, 1.25 mmol) in 3.0  
10 mL of THF was cooled to -78 °C and *tert*-butyllithium (160.8 mg, 2.51 mmol,  
11 1.5 ml of a 1.7M solution in pentane) was added to give a yellow solution.  
12 After 30 minutes a solution of ZnCl<sub>2</sub> (345.0 mg, 2.53 mmol) in 5.0 mL THF  
13 was slowly added via cannula. The resulting solution was warmed to room  
14 temperature and transferred via cannula to a solution of ethyl 4-((2,2-  
15 dimethyl-4-trifluoromethanesulfonyloxy-(2H)-chromen-6-yl)ethynyl)-benzoate  
16 (Compound 264, 150.0 mg, 0.31 mmol) and tetrakis(triphenylphosphine)  
17 palladium(0) (24.0 mg, 0.02 mmol) in 2.0 mL THF. This solution was heated  
18 to 50 °C for 2 hours, cooled to room temperature, and the reaction quenched  
19 by the addition of saturated aqueous NH<sub>4</sub>Cl. This solution was extracted with  
20 EtOAc and the combined organic layers were washed with H<sub>2</sub>O and saturated  
21 aqueous NaCl before being dried (MgSO<sub>4</sub>) and concentrated under reduced  
22 pressure. The title compound, 100.0 mg (72%), was isolated by column  
23 chromatography (2.5% EtOAc / hexanes) as a colorless solid. <sup>1</sup>H NMR (300  
24 MHz, CDCl<sub>3</sub>) δ: 7.99 (2H, d, J = 8.3 Hz), 7.52 (2H, d, J = 8.3 Hz), 7.36 (1H,  
25 dd, J = 2.1, 8.3 Hz), 7.30-7.26 (5H, m), 6.88 (1H, d, J = 8.3 Hz), 5.64 (1H,  
26 s), 4.38 (2H, q, J = 7.1 Hz), 2.98 (1H, hept, J = 7.0 Hz), 1.51 (6H, s), 1.40  
27 (3H, t, J = 7.1 Hz), 1.31 (6H, d, J = 7.0 Hz).

28 Ethyl 4-[[4-(4-(1,1-dimethylethyl)phenyl)-2,2-dimethyl-(2H)-chromen-6-yl]-  
29 ethynyl]-benzoate (Compound 269)

1 A solution of 4-*tert*-butylbromobenzene (280.0 mg, 1.61 mmol) in 3.0  
2 mL of THF was cooled to -78 °C and *tert*-butyllithium (206.3 mg, 3.22 mmol,  
3 1.7 ml of a 1.7M solution in pentane) was added to give a yellow solution.  
4 After 30 minutes a solution of ZnCl<sub>2</sub> (380.0 mg, 3.22 mmol) in 5.0 mL THF  
5 was slowly added via cannula. The resulting solution was warmed to room  
6 temperature and transferred via cannula to a solution of ethyl 4-((2,2-  
7 dimethyl-4-trifluoromethanesulfonyloxy-(2H)-chromen-6-yl)ethynyl)-benzoate  
8 (Compound 264, 150.0 mg, 0.31 mmol) and tetrakis(triphenylphosphine)  
9 palladium(0) (24.0 mg, 0.02 mmol) in 2.0 mL THF. This solution was heated  
10 to 50 °C for 2 hours, cooled to room temperature, and the reaction quenched  
11 by the addition of saturated aqueous NH<sub>4</sub>Cl. This solution was extracted with  
12 EtOAc and the combined organic layers were washed with H<sub>2</sub>O and saturated  
13 aqueous NaCl before being dried (MgSO<sub>4</sub>) and concentrated under reduced  
14 pressure. The title compound, 85.0 mg (59%), was isolated by column  
15 chromatography (2.5% EtOAc / hexanes) as a colorless solid. <sup>1</sup>H NMR (300  
16 MHz, CDCl<sub>3</sub>) δ: 7.99 (2H, d, J = 8.3 Hz), 7.53 (2H, d, J = 8.3 Hz), 7.45 (1H,  
17 d, J = 8.4 Hz), 7.37 (1H, dd, J = 2.0, 8.3 Hz), 7.32-7.28 (3H, m), 6.89 (1H, d,  
18 J = 8.3 Hz), 5.66 (1H, s), 4.39 (2H, q, J = 7.1 Hz), 1.52 (6H, s), 1.40 (3H, t,  
19 J = 7.1 Hz), 1.39 (9H, s).

20 Ethyl 2-fluoro-4-[(2,2-dimethyl-4-oxo-chroman-6-yl)ethynyl]-benzoate  
21 (Compound 270)

22 A solution of 6-ethynyl-2,2-dimethylchroman-4-one (Compound 262,  
23 314.0 mg, 1.57 mmol) and ethyl 2-fluoro-4-iodobenzoate (440.0 mg, 1.50  
24 mmol) in 10.0 mL Et<sub>3</sub>N was purged with argon for 15 minutes. To this  
25 solution was added bis(triphenylphosphine)-palladium(II) chloride (275.0 mg,  
26 0.39 mmol) and copper(I) iodide (75.0 mg, 0.39 mmol). After sparging for an  
27 additional 10 minutes with argon, the solution was stirred overnight at room  
28 temperature. The reaction mixture was filtered through a pad of Celite using  
29 an Et<sub>2</sub>O wash. Concentration of the filtrate under reduced pressure, followed



1 by column chromatography (3-5% EtOAc-hexanes) of the residual solid  
2 afforded 400.0 mg (69%) the title compound as an orange solid. <sup>1</sup>H NMR  
3 (300 MHz, CDCl<sub>3</sub>) δ : 8.05 (1H, d, J = 2.1 Hz), 7.90 (1H, d, J = 7.8 Hz),  
4 7.60 (1H, dd, J = 2.2 8.5 Hz), 7.29 (1H, dd, J = 1.5, 8.2 Hz), 7.25 (1H, d, J  
5 = 11.4 Hz), 6.93 (1H, d, J = 8.5 Hz), 4.39 (2H, q, J = 7.1 Hz), 2.75 (2H, s),  
6 1.48 (6H, s), 1.40 (3H, t, J = 7.1 Hz).

7 Ethyl 2-fluoro-4-((2,2-dimethyl-4-trifluoromethanesulfonyloxy-(2H)-chromen-  
8 6-yl)ethynyl)-benzoate (Compound 271)

9 A solution of sodium bis(trimethylsilyl)amide (238.0 mg, 1.30 mmol) in  
10 3.0 mL of THF was cooled to -78 °C and a solution of ethyl 2-fluoro-4-((2,2-  
11 dimethyl-4-oxo-chroman-6-yl)ethynyl)-benzoate (Compound 270, 400.0 mg,  
12 1.09 mmol) in 2.0 mL was added slowly. After 30 minutes a solution of 2-  
13 [N,N-bis(trifluoromethanesulfonyl)amino]-5-pyridine (510.0 mg, 1.30 mmol)  
14 in 1.0 mL of THF was added. After 5 minutes the solution was warmed to  
15 room temperature and stirred overnight. The reaction was quenched by the  
16 addition of saturated aqueous NH<sub>4</sub>Cl and extracted with EtOAc. The  
17 combined organic layers were washed with 5% aqueous NaOH and H<sub>2</sub>O  
18 before being dried (MgSO<sub>4</sub>) and concentrated under reduced pressure. The  
19 title compound, 315.0 mg (58%), was isolated by column chromatography  
20 (5% EtOAc / hexanes) as a colorless solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ :  
21 7.92 (1H, d, J = 7.8 Hz), 7.44-7.26 (4H, m), 6.85 (1H, d, J = 8.7 Hz), 5.70  
22 (1H, s), 4.31 (2H, q, J = 7.1 Hz), 1.55 (6H, s), 1.41 (3H, t, J = 7.1 Hz).

23 Ethyl 2-fluoro-4-[[4-(4-methylphenyl)-2,2-dimethyl-(2H)-chromen-6-yl]-  
24 ethynyl]-benzoate (Compound 272)

25 A solution of 4-methylbromobenzene (140.0 mg, 0.80 mmol) in 3.0 mL  
26 of THF was cooled to -78 °C and *tert*-butyllithium (102.5 mg, 1.60 mmol, 0.94  
27 mL of a 1.7M solution in pentane) was added to give a yellow solution. After  
28 30 minutes a solution of ZnCl<sub>2</sub> (174.0 mg, 1.28 mmol) in 5.0 mL THF was  
29 slowly added via cannula. The resulting solution was warmed to room

1 temperature and transferred via cannula to a solution of ethyl 2-fluoro-4-(2,2-  
2 dimethyl-4-trifluoromethanesulfonyloxy-(2H)-chromen-6-ylethynyl)-benzoate  
3 (Compound 271, 150.0 mg, 0.31 mmol) and  
4 tetrakis(triphenylphosphine)palladium(0) (20.0 mg, 0.02 mmol) in 2.0 mL  
5 THF. This solution was heated to 50 °C for 2 hours; cooled to room  
6 temperature, and the reaction quenched by the addition of saturated aqueous  
7 NH<sub>4</sub>Cl. This solution was extracted with EtOAc and the combined organic  
8 layers were washed with H<sub>2</sub>O and saturated aqueous NaCl before being dried  
9 (MgSO<sub>4</sub>) and concentrated under reduced pressure. The title compound,  
10 86.0 mg (62%), was isolated by column chromatography (5% EtOAc /  
11 hexanes) as a colorless solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 7.88 (1H, d, J =  
12 7.8 Hz), 7.35 (1H, d, J = 7.8 Hz), 7.35 (1H, dd, J = 2.0, 8.4 Hz), 7.28-7.19  
13 (7H, m), 6.88 (1H, d, J = 8.3 Hz), 5.64 (1H, s), 4.40 (2H, q, J = 7.1 Hz), 2.43  
14 (3H, s), 1.51 (6H, s), 1.41 (3H, t, J = 7.1 Hz).

15 Ethyl 2-fluoro-4-[[4-(4-ethylphenyl)-2,2-dimethyl-(2H)-chromen-6-yl]-ethynyl]-  
16 benzoate (Compound 273)

17 A solution of 4-ethylbromobenzene (150.0 mg, 0.80 mmol) in 3.0 mL  
18 of THF was cooled to -78 °C and *tert*-butyllithium (102.5 mg, 1.60 mmol, 0.94  
19 ml of a 1.7M solution in pentane) was added to give a yellow solution. After  
20 30 minutes a solution of ZnCl<sub>2</sub> (218.0 mg, 1.60 mmol) in 5.0 mL THF was  
21 slowly added via cannula. The resulting solution was warmed to room  
22 temperature and transferred via cannula to a solution of ethyl 2-fluoro-4-(2,2-  
23 dimethyl-4-trifluoromethanesulfonyloxy-(2H)-chromen-6-ylethynyl)-benzoate  
24 (Compound 271, 160.0 mg, 0.32 mmol) and  
25 tetrakis(triphenylphosphine)palladium(0) (20.0 mg, 0.02 mmol) in 2.0 mL  
26 THF. This solution was heated to 50 °C for 2 hours, cooled to room  
27 temperature, and the reaction quenched by the addition of saturated aqueous  
28 NH<sub>4</sub>Cl. This solution was extracted with EtOAc and the combined organic  
29 layers were washed with H<sub>2</sub>O and saturated aqueous NaCl before being dried

(MgSO<sub>4</sub>) and concentrated under reduced pressure. The title compound, 115.0 mg (79%), was isolated by column chromatography (5% EtOAc / hexanes) as a colorless solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 7.88 (1H, d, J = 7.8 Hz), 7.35 (1H, dd, J = 2.0, 8.3 Hz), 7.28-7.20 (7H, m), 6.88 (1H, d, J = 8.3 Hz), 5.65 (1H, s), 4.39 (2H, q, J = 7.1 Hz), 2.73 (2H, q, J = 7.6 Hz), 1.52 (6H, s), 1.40 (3H, t, J = 7.1 Hz), 1.31 (3H, t, J = 7.6 Hz).

4-[[4-phenyl-2,2-dimethyl-(2H)-chromen-6-yl]-ethynyl]-benzoic acid  
(Compound 274)

To a solution of ethyl 4-[[4-phenyl-2,2-dimethyl-(2H)-chromen-6-yl]-ethynyl]-benzoate (Compound 265, 70.0 mg, 0.171 mmol) in 3.0 mL THF and 3.0 mL EtOH was added NaOH (120.0 mg, 3.0 mmol, 3.0 mL of a 1M aqueous solution). The resulting solution was heated to 35 °C, cooled to room temperature and stirred overnight. The reaction was acidified with 10% aqueous HCl and extracted with EtOAc. The combined organic layers were washed with H<sub>2</sub>O, saturated aqueous NaCl, and dried (Na<sub>2</sub>SO<sub>4</sub>) before the solvent was removed under reduced pressure. The residual solid was recrystallized from CH<sub>3</sub>CN to give 48.0 mg (74%) of the title compound as a colorless solid. <sup>1</sup>H NMR (300 MHz, d<sub>6</sub>-acetone) δ: 8.00 (2H, d, J = 8.2 Hz), 7.57 (2H, d, J = 8.2 Hz), 7.51-7.37 (6H, d), 7.14 (1H, d, J = 2.0 Hz), 6.91 (1H, d, J = 8.3 Hz), 5.80 (1H, s), 1.50 (6H, s),

4-[[4-(4-methylphenyl)-2,2-dimethyl-(2H)-chromen-6-yl]-ethynyl]-benzoic acid  
(Compound 275)

To a solution of ethyl 4-[[4-(4-methylphenyl)-2,2-dimethyl-(2H)-chromen-6-yl]-ethynyl]-benzoate (Compound 266, 90.0 mg, 0.213 mmol) in 3.0 mL THF and 3.0 mL EtOH was added NaOH (120.0 mg, 3.0 mmol, 3.0 mL of a 1M aqueous solution). The resulting solution was heated to 35 °C, cooled to room temperature and stirred overnight. The reaction mixture was acidified with 10% aqueous HCl and extracted with EtOAc. The combined organic layers were washed with H<sub>2</sub>O, saturated aqueous NaCl, and dried

1 (Na<sub>2</sub>SO<sub>4</sub>) before the solvent was removed under reduced pressure. The  
2 residual solid was recrystallized from CH<sub>3</sub>CN to give 70.0 mg (83%) the title  
3 compound as a colorless solid. <sup>1</sup>H NMR (300 MHz, d<sub>6</sub>-acetone) δ: 8.01 (2H,  
4 d, J = 8.3 Hz), 7.57 (2H, d, J = 8.3 Hz), 7.40 (1H, dd, J = 2.1, 8.3 Hz), 7.30-  
5 7.20 (4H, m), 7.16 (1H, d, J = 2.0 Hz), 6.90 (1H, d, J = 8.3 Hz), 5.67 (1H, s),  
6 2.38 (3H, s), 1.49 (6H, s).

7 4-[[4-(4-ethylphenyl)-2,2-dimethyl-(2H)-chromen-6-yl]-ethynyl]-benzoic acid  
8 (Compound 276)

9 To a solution of ethyl 4-[[4-(4-ethylphenyl)-2,2-dimethyl-(2H)-chromen-  
10 6-yl]-ethynyl]-benzoate (Compound 267, 82.0 mg, 0.188 mmol) in 3.0 mL  
11 THF and 3.0 mL EtOH was added NaOH (120.0 mg, 3.0 mmol, 3.0 mL of a  
12 1M aqueous solution). The resulting solution was heated to 35 °C, cooled to  
13 room temperature and stirred overnight. The reaction mixture was acidified  
14 with 10% aqueous HCl and extracted with EtOAc. The combined organic  
15 layers were washed with H<sub>2</sub>O, saturated aqueous NaCl, and dried (Na<sub>2</sub>SO<sub>4</sub>)  
16 before the solvent was removed under reduced pressure. The residual solid  
17 was recrystallized from CH<sub>3</sub>CN to give 65.0 mg (85%) the title compound as  
18 a colorless solid. <sup>1</sup>H NMR (300 MHz, d<sub>6</sub>-acetone) δ: 8.01 (2H, d, J = 8.4 Hz),  
19 7.57 (2H, d, J = 8.4 Hz), 7.40 (1H, dd, J = 2.0, 8.3 Hz), 7.35-7.25 (4H, m),  
20 7.17 (1H, d, J = 2.0 Hz), 6.90 (1H, d, J = 8.3 Hz), 5.77 (1H, s), 2.69 (2H, q, J  
21 = 7.6 Hz), 1.49 (6H, s), 1.24 (2H, t, J = 7.6 Hz).

22 4-[[4-(4-(1-methylethyl)phenyl)-2,2-dimethyl-(2H)-chromen-6-yl]-ethynyl]-  
23 benzoic acid (Compound 277)

24 To a solution of ethyl 4-[[4-(4-(1-methylethyl)phenyl)-2,2-dimethyl-  
25 (2H)-chromen-6-yl]-ethynyl]-benzoate (Compound 268, 95.0 mg, 0.210 mmol)  
26 in 3.0 mL THF and 3.0 mL EtOH was added NaOH (120.0 mg, 3.0 mmol,  
27 3.0 mL of a 1M aqueous solution). The resulting solution was heated to 35  
28 °C, cooled to room temperature and stirred overnight. The reaction mixture  
29 was acidified with 10% aqueous HCl and extracted with EtOAc. The

1 combined organic layers were washed with H<sub>2</sub>O, saturated aqueous NaCl, and  
2 dried (Na<sub>2</sub>SO<sub>4</sub>) before the solvent was removed under reduced pressure. The  
3 residual solid was recrystallized from CH<sub>3</sub>CN to give 75.0 mg (84%) the title  
4 compound as a colorless solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 8.05 (2H, d, J  
5 = 8.4 Hz), 7.55 (2H, d, J = 8.4 Hz), 7.36 (1H, dd, J = 2.0, 8.3 Hz), 7.30-7.26  
6 (5H, m), 6.88 (1H, d, J = 8.3 Hz), 5.64 (1H, s), 2.98 (1H, hept, J = 6.9 Hz),  
7 1.51 (6H, s), 1.31 (6H, d, J = 6.9 Hz).

8 4-[[4-(4-(1,1-dimethylethyl)phenyl)-2,2-dimethyl-(2H)-chromen-6-yl]-ethynyl]-  
9 benzoic acid (Compound 278)

10 To a solution of ethyl 4-[[4-(4-(1,1-dimethylethyl)phenyl)-2,2-dimethyl-  
11 (2H)-chromen-6-yl]-ethynyl]-benzoate (Compound 269, 72.0 mg, 0.155 mmol)  
12 in 3.0 mL THF and 3.0 mL EtOH was added NaOH (120.0 mg, 3.0 mmol,  
13 3.0 mL of a 1M aqueous solution). The resulting solution was heated to 35  
14 °C, cooled to room temperature and stirred overnight. The reaction mixture  
15 was acidified with 10% aqueous HCl and extracted with EtOAc. The  
16 combined organic layers were washed with H<sub>2</sub>O, saturated aqueous NaCl, and  
17 dried (Na<sub>2</sub>SO<sub>4</sub>) before the solvent was removed under reduced pressure. The  
18 residual solid was recrystallized from CH<sub>3</sub>CN to give 50.0 mg (74%) the title  
19 compound as a colorless solid. <sup>1</sup>H NMR (300 MHz, d<sub>6</sub>-acetone) δ: 8.00 (2H,  
20 d, J = 8.3 Hz), 7.57 (2H, d, J = 8.3 Hz), 7.51 (2H, d, J = 8.3 Hz), 7.41 (1H,  
21 dd, J = 2.0, 8.3 Hz), 7.31 (2H, d, J = 8.3 Hz), 7.19 (1H, d, J = 2.0 Hz), 6.91  
22 (1H, d, J = 8.3 Hz), 5.78 (1H, s), 1.49 (6H, s), 1.35 (9H, s).

23 2-Fluoro-4-[[4-(4-methylphenyl)-2,2-dimethyl-(2H)-chromen-6-yl]-ethynyl]-  
24 benzoic acid (Compound 279)

25 To a solution of ethyl 2-fluoro-4-[[4-(4-methylphenyl)-2,2-dimethyl-  
26 (2H)-chromen-6-yl]-ethynyl]-benzoate (Compound 272, 60.0 mg, 0.136 mmol)  
27 in 2.0 mL THF and 1.0 mL EtOH was added NaOH (120.0 mg, 3.0 mmol,  
28 3.0 mL of a 1M aqueous solution). The resulting solution was heated to 35  
29 °C, cooled to room temperature and stirred overnight. The reaction mixture

1 was acidified with 10% aqueous HCl and extracted with EtOAc. The  
2 combined organic layers were washed with H<sub>2</sub>O, saturated aqueous NaCl, and  
3 dried (Na<sub>2</sub>SO<sub>4</sub>) before the solvent was removed under reduced pressure. The  
4 residual solid was recrystallized from CH<sub>3</sub>CN to give 53.0 mg (94%) the title  
5 compound as a colorless solid. <sup>1</sup>H NMR (300 MHz, d<sub>6</sub>-acetone) δ: 7.93 (1H,  
6 d, J = 7.8 Hz), 7.43-7.16 (8H, m), 6.91 (1H, d, J = 8.3 Hz), 5.77 (1H, s), 2.38  
7 (3H, s), 1.49 (6H, s).

8 2-Fluoro-4-[[4-(4-ethylphenyl)-2,2-dimethyl-(2H)-chromen-6-yl]-ethynyl]-  
9 benzoic acid (Compound 280)

10 To a solution of ethyl 2-fluoro-4-[[4-(4-ethylphenyl)-2,2-dimethyl-(2H)-  
11 chromen-6-yl]-ethynyl]-benzoate (Compound 273, 82.0 mg, 0.180 mmol) in  
12 2.0 mL THF and 2.0 mL EtOH was added NaOH (120.0 mg, 3.0 mmol, 3.0  
13 mL of a 1M aqueous solution). The resulting solution was heated to 35 °C,  
14 cooled to room temperature and stirred overnight. The reaction mixture was  
15 acidified with 10% aqueous HCl and extracted with EtOAc. The combined  
16 organic layers were washed with H<sub>2</sub>O, saturated aqueous NaCl, and dried  
17 (Na<sub>2</sub>SO<sub>4</sub>) before the solvent was removed under reduced pressure. The  
18 residual solid was recrystallized from CH<sub>3</sub>CN to give 70.0 mg (91%) the title  
19 compound as a pale-yellow solid. <sup>1</sup>H NMR (300 MHz, d<sub>6</sub>-acetone) δ: 7.93  
20 (1H, d, J = 7.8 Hz), 7.41 (1H, dd, J = 2.1, 8.3 Hz), 7.37-7.25 (6H, m), 7.18  
21 (1H, d, J = 2.0 Hz), 6.91 (1H, d, J = 8.3 Hz), 5.77 (1H, s), 2.69 (2H, q, J =  
22 7.6 Hz), 1.49 (6H, s), 1.24 (3H, t, J = 7.6 Hz).

23 Method of Potentiating Nuclear Receptor Agonists

24 Overview and introduction

25 We have discovered that a subset of retinoid antagonists which exhibit  
26 negative hormone activity can be used for potentiating the biological activities  
27 of other retinoids and steroid receptor superfamily hormones. These other  
28 retinoids and steroid receptor superfamily hormones can be either  
29 endogenous hormones or pharmaceutical agents. Thus, for example, when

1 used in combination with a retinoid negative hormone, certain activities of  
2 pharmaceutical retinoid agonists can be rendered more active in eliciting  
3 specific biological effects. Advantageously, this combination approach to  
4 drug administration can minimize undesirable side effects of pharmaceutical  
5 retinoids because lower dosages of the pharmaceutical retinoids can be used  
6 with improved effectiveness.

7 More particularly, we have discovered that AGN 193109, a synthetic  
8 retinoid having the structure shown in Figure 1, exhibits unique and  
9 unexpected pharmacologic activities. AGN 193109 exhibits high affinity for  
10 the RAR subclass of nuclear receptors without activating these receptors or  
11 stimulating transcription of retinoid responsive genes. Instead, AGN 193109  
12 inhibits the activation of RARs by retinoid agonists and therefore behaves as  
13 a retinoid antagonist.

14 Additionally, we have discovered that retinoid negative hormones can  
15 be used without coadministration of a retinoid agonist or steroid hormone to  
16 control certain disease symptoms. More specifically, the retinoid negative  
17 hormone disclosed herein can down-regulate the high level basal transcription  
18 of genes that are responsive to unliganded RARs. If, for example,  
19 uncontrolled cellular proliferation results from the activity of genes  
20 responsive to unliganded RARs, then that gene activity can be reduced by the  
21 administration of a retinoid negative hormone that inactivates RARs.  
22 Consequently, cellular proliferation dependent on the activity of unliganded  
23 RARs can be inhibited by the negative hormone. Inhibition of unliganded  
24 RARs cannot be achieved using conventional antagonists.

25 Significantly, we have discovered that AGN 193109 can both repress  
26 RAR basal activity and can sometimes potentiate the activities of other  
27 retinoid and steroid-receptor-superfamily hormone agonists. In the context  
28 of the invention, a hormone agonist is said to be potentiated by a negative  
29 hormone such as AGN 193109 if, in the presence of the negative hormone, a

1 reduced concentration of the agonist elicits substantially the same  
2 quantitative response as that obtainable with the agonist alone. The  
3 quantitative response can, for example, be measured in a reporter gene assay  
4 *in vitro*. Thus, a therapeutic retinoid that elicits a desired response when  
5 used at a particular dosage or concentration is potentiated by AGN 193109 if,  
6 in combination with AGN 193109, a lower dosage or concentration of the  
7 therapeutic retinoid can be used to produce substantially the same effect as a  
8 higher dosage or concentration of the therapeutic retinoid when that  
9 therapeutic retinoid is used alone. The list of agonists that can be  
10 potentiated by coadministration with AGN 193109 includes RAR agonists,  
11 vitamin D receptor agonists, glucocorticoid receptor agonists and thyroid  
12 hormone receptor agonists. More particularly, specific agonists that can be  
13 potentiated by coadministration include: ATRA, 13-cis retinoic acid, the  
14 synthetic RAR agonist AGN 191183, 1,25-dihydroxyvitamin D<sub>3</sub>,  
15 dexamethasone and thyroid hormone (3,3',5-triiodothyronine). Also disclosed  
16 herein is a method that can be used to identify other hormones that can be  
17 potentiated by coadministration with AGN 193109.

18 Thus, AGN 193109 behaves in a manner not anticipated for a simple  
19 retinoid antagonist, but as a negative hormone that can potentiate the  
20 activities of various members of the family of nuclear receptors. We also  
21 disclose a possible mechanism that can account for both negative hormone  
22 activity and the ability of AGN 193109 to potentiate the activities of other  
23 nuclear receptor ligands. This mechanism incorporates elements known to  
24 participate in retinoid-dependent signalling pathways and additionally  
25 incorporates a novel negative regulatory component.

26 Those having ordinary skill in the art will appreciate that RARs, which  
27 are high-affinity targets of AGN 193109 binding, are transcription factors that  
28 regulate the expression of a variety of retinoid responsive genes. Cis-  
29 regulatory DNA binding sites for the RARs have been identified nearby



1 genes that are transcriptionally regulated in a retinoid-dependent fashion.  
2 RAR binding to such DNA sites, known as retinoic acid response elements  
3 (RAREs), has been well defined. Importantly, the RAREs bind  
4 heterodimers consisting of one RAR and one RXR. The RXR component  
5 of the heterodimer functions to promote a high affinity interaction between  
6 the RAR/RXR heterodimer and the RARE (Mangelsdorf et al. *The Retinoid*  
7 *Receptors in The Retinoids: Biology, Chemistry and Medicine*, 2nd edition,  
8 eds. Sporn et al., Raven Press, Ltd., New York 1994, the disclosure of which  
9 is hereby incorporated by reference).

10 As detailed below, our findings related to the negative hormone  
11 activity of AGN 193109 are consistent with a mechanism involving the  
12 interaction of a putative Negative Coactivator Protein (NCP) with the RAR.  
13 According to the proposed mechanism, this interaction is stabilized by AGN  
14 193109.

15 Our results further indicated that AGN 193109 can modulate  
16 intracellular availability of NCP for interaction with nuclear receptors other  
17 than RARs that are occupied by AGN 193109. It follows that AGN 193109  
18 can potentiate transcriptional regulatory pathways involving nuclear receptors  
19 that share with the RARs the ability to bind the NCP. In this regard, AGN  
20 193109 exhibits the ability to modulate a variety of nuclear receptor pathways,  
21 an activity that would not be predicted for a conventional retinoid antagonist.  
22 Accordingly, AGN 193109 is useful as an agent for potentiating the activity of  
23 nuclear receptor ligands, including both endogenous hormones and  
24 prescribed therapeutics. This specific embodiment illustrates the more  
25 general principle that any nuclear receptor negative hormone will potentiate  
26 the activity of other nuclear receptors that competitively bind the NCP.

27 Although other materials and methods similar or equivalent to those  
28 described herein can be used in the practice or testing of the present  
29 invention, the preferred methods and materials are now described. General

1 references for methods that can be used to perform the various nucleic acid  
2 manipulations and procedures described herein can be found in *Molecular*  
3 *Cloning: A Laboratory Manual* (Sambrook et al. eds. Cold Spring Harbor Lab  
4 Publ. 1989) and *Current Protocols in Molecular Biology* (Ausubel et al. eds.,  
5 Greene Publishing Associates and Wiley-Interscience 1987). The disclosures  
6 contained in these references are hereby incorporated by reference. A  
7 description of the experiments and results that led to the creation of the  
8 present invention follows.

9 Example 6 describes the methods used to demonstrate that AGN  
10 193109 bound each of three RARs with high affinity but failed to activate  
11 retinoid dependent gene expression.

#### 12 Example 6

#### 13 AGN 193109 Binds RARs With High Affinity But Does Not

#### 14 Transactivate Retinoid-Dependent Gene Expression

15 Human RAR- $\alpha$ , RAR- $\beta$  and RAR- $\gamma$  receptors were separately  
16 expressed as recombinant proteins using a baculovirus expression system  
17 essentially according to the method described by Allegretto et al. in *J. Biol.*  
18 *Chem.* 268:26625 (1993). The recombinant receptor proteins were separately  
19 employed for determining AGN 193109 binding affinities using the [ $^3\text{H}$ ]-  
20 ATRA displacement assay described by Heyman et al. in *Cell* 68:397 (1992).  
21 Dissociation constants (Kds) were determined according to the procedure  
22 described by Cheng et al. in *Biochemical Pharmacology* 22:3099 (1973).

23 AGN 193109 was also tested for its ability to transactivate RARs in  
24 CV-1 cells transiently cotransfected with RAR expression vectors and a  
25 retinoid responsive reporter gene construct. Receptor expression vectors  
26 pRShRAR- $\alpha$  (Giguere et al. *Nature* 330:624 (1987)), pRShRAR- $\beta$  (Benbrook  
27 et al. *Nature* 333:669 (1988)) and pRShRAR- $\gamma$  (Ishikawa et al. *Mol.*  
28 *Endocrinol.* 4:837 (1990)) were separately cotransfected with the  $\Delta\text{MTV-}$   
29 TREp-Luc reporter plasmid. Use of this luciferase reporter plasmid has been

1 disclosed by Heyman et al. in *Cell* 68:397 (1992). The  $\Delta$ MTV-TREp-Luc  
2 plasmid is essentially identical to the  $\Delta$ MTV-TREp-CAT reporter construct  
3 described by Umesono et al. in *Nature* 336:262 (1988), except that the  
4 chloramphenicol acetyltransferase (CAT) reporter gene was substituted by a  
5 polynucleotide sequence encoding firefly luciferase. Transfection of green  
6 monkey CV-1 cells was carried out using the calcium phosphate  
7 coprecipitation method described in *Molecular Cloning: A Laboratory Manual*  
8 (Sambrook et al. eds. Cold Spring Harbor Lab Publ. 1989). CV-1 cells were  
9 plated at a density of  $4 \times 10^4$ /well in 12 well multiwell plates and transiently  
10 transfected with a calcium phosphate precipitate containing  $0.7 \mu\text{g}$  of reporter  
11 plasmid and  $0.1 \mu\text{g}$  of receptor plasmid according to standard laboratory  
12 procedures. Cells were washed after 18 hours to remove the precipitate and  
13 refed with Dulbecco's modified Eagle's medium (DMEM) (Gibco),  
14 containing 10% activated charcoal extracted fetal bovine serum (Gemini Bio-  
15 Products). Cells were treated with vehicle alone (ethanol) or AGN 193109  
16 ( $10^{-9}$  to  $10^{-6}$  M) for 18 hours. Cell lysates were prepared in  $0.1 \text{ M KPO}_4$  (pH  
17 7.8), 1.0% TRITON X-100, 1.0 mM DTT, 2 mM-EDTA. Luciferase activity  
18 was measured as described by de Wet et al. in *Mol. Cell. Biol.* 7:725 (1987),  
19 using firefly luciferin (Analytical Luminescence Laboratory) and an EG&G  
20 Berthold 96-well plate luminometer. Reported luciferase values represented  
21 the mean  $\pm$  SEM of triplicate determinations.

22 The results presented in Table 11 indicated that AGN 193109 bound  
23 each of RAR- $\alpha$ , RAR- $\beta$  and RAR- $\gamma$  with high affinity, but did not activate  
24 retinoid-dependent gene expression. More specifically, AGN 193109 bound  
25 each of the three receptors with  $K_d$  values in the 2 - 3 nM range. Despite  
26 this tight binding, AGN 193109 failed to activate gene expression when  
27 compared with inductions stimulated by ATRA. Accordingly, the half-  
28 maximal effective concentration of AGN 193109 ( $\text{EC}_{50}$ ) was unmeasurable.  
29 Although not presented in the Table, we also found that AGN 193109 had no

1 measurable affinity for the RXRs.

2 TABLE 11

3 AGN 193109 Binding and Transactivation of the RARs

4	RAR- $\alpha$	RAR- $\beta$	RAR- $\gamma$
5 EC <sub>50</sub> (nM)	No Activity	No Activity	No Activity
6 K <sub>d</sub> (nM)	2	2	3

7  
8 Example 7 describes the methods used to demonstrate that AGN  
9 193109 is an antagonist of ATRA dependent gene expression.

10 Example 7

11 AGN 193109-Dependent Inhibition of RAR Transactivation  
12 by ATRA

13 The ability of AGN 193109 to antagonize ATRA mediated RAR  
14 activation was investigated in CV-1 cells cotransfected by the calcium  
15 phosphate coprecipitation method of Sambrook et al. (*Molecular Cloning: A*  
16 *Laboratory Manual* Cold Spring Harbor Lab Publ. 1989). Eukaryotic  
17 expression vectors pRShRAR- $\alpha$  (Giguere et al. *Nature* 330:624 (1987)),  
18 pRShRAR- $\beta$  (Benbrook et al. *Nature* 333:669 (1988)) and pRShRAR- $\gamma$   
19 (Ishikawa et al. *Mol. Endocrinol.* 4:837 (1990)) were cotransfected with the  $\Delta$ -  
20 MTV-Luc reporter plasmid described by Hollenberg et al. (*Cell* 55:899  
21 (1988)). Notably, the reporter plasmid contained two copies of the TRE-  
22 palindromic response element. Calcium phosphate transfections were carried  
23 out exactly as described in Example 6. Cells were dosed with vehicle alone  
24 (ethanol), ATRA ( $10^{-9}$  to  $10^{-6}$  M), AGN 193109 ( $10^{-9}$  to  $10^{-6}$  M), or  $10^{-8}$  M  
25 ATRA in combination with AGN 193109 ( $10^{-9}$  to  $10^{-6}$  M) for 18 hours. Cell  
26 lysates and luciferase activity measurements were also performed as in  
27 Example 6.

28 The results of these procedures are presented in Figures 2A through  
29 2F where luciferase values represent the mean  $\pm$  SEM of triplicate  
30 determinations. More specifically, the results presented in Figures 2A, 2C

1 and 2E indicated that stimulation of transfected cells with ATRA led to dose  
2 responsive increases in luciferase activity. This confirmed that ATRA  
3 activated each of the three RARs in the experimental system and provided a  
4 comparative basis for detecting the activity of an antagonist. The graphic  
5 results presented in Figures 2B, 2D and 2F indicated that cotreatment of  
6 transfected cells with 10 nM ATRA and increasing concentrations of AGN  
7 193109 led to an inhibition of luciferase activity. In particular, equal doses of  
8 AGN 193109 and ATRA gave greater than 50% inhibition relative to ATRA  
9 alone for all three RAR subtypes. Comparison of the ATRA dose response  
10 in the presence of different concentrations of AGN 193109 indicated that  
11 ATRA was competitively inhibited by AGN 193109. Notably, the horizontal  
12 axes on all of the graphs shown in Figure 2 represents the log of the retinoid  
13 concentration. These results proved that AGN 193109 was a potent RAR  
14 antagonist.

15 We next performed experiments to elucidate the mechanism underlying  
16 the antagonist activity of AGN 193109. Those having ordinary skill in the art  
17 will appreciate that nuclear receptor activation is believed to involve a  
18 conformational change of the receptor that is induced by ligand binding.  
19 Indeed, the results of protease protection assays have confirmed that nuclear  
20 hormone agonists and antagonists cause receptor proteins to adopt different  
21 conformations (Keidel et al. *Mol. Cell. Biol.* 14:287 (1994); Allan et al. *J. Biol.*  
22 *Chem.* 267:19513 (1992)). We used such an assay to determine if AGN  
23 193109 and ATRA caused RAR- $\alpha$  to adopt different conformations. AGN  
24 193583, an RAR- $\alpha$ -selective antagonist, was included as a positive control  
25 that is known to confer an antagonist-specific pattern of protease sensitivity.

26 Example 8 describes one method that was used to detect  
27 conformational changes in RAR- $\alpha$  resulting from AGN 193109 binding. As  
28 presented below, the results of this procedure unexpectedly indicated that  
29 AGN 193109 led to a pattern of trypsin sensitivity that was substantially

1 identical to that induced by ATRA, an RAR agonist, and unlike that induced  
2 by a model RAR antagonist. This finding suggested that AGN 193109  
3 possessed properties distinct from other retinoid antagonists.

#### 4 **Example 8**

##### 5 Protease Protection Analysis

6 A plasmid constructed in the vector pGEM3Z (Pharmacia) and  
7 containing the RAR- $\alpha$  cDNA (Giguere et al. *Nature* 330:624 (1987)), was  
8 used in connection with the TNT-coupled reticulocyte lysate *in vitro*  
9 transcription-translation system (Promega) to prepare [ $^{35}$ S]-methionine  
10 labeled RAR- $\alpha$ . Limited proteolytic digestion of the labeled protein RAR- $\alpha$   
11 was carried out according to the method described by Keidel et al. in *Mol.*  
12 *Cell. Biol.* 14:287 (1994). Aliquots of reticulocyte lysate containing [ $^{35}$ S]-  
13 methionine labeled RAR- $\alpha$  were incubated with either ATRA, AGN 193583  
14 or AGN 193109 on ice for 45 minutes in a total volume of 9  $\mu$ l. The retinoid  
15 final concentration for all trials was 100 nM for ATRA and AGN 193109,  
16 and 1000 nM for AGN 193583. The difference between the final  
17 concentrations of the retinoids was based on the approximate 10-fold  
18 difference in relative affinities of ATRA and AGN 193109 (having Kd at  
19 RAR- $\alpha$  of 2 and 10 nM, respectively) and AGN 193583 (having Kd at RAR-  
20  $\alpha$  of  $\geq 100$  nM). After ligand binding, 1  $\mu$ l of appropriately concentrated  
21 trypsin was added to the mixture to give final concentrations of 25, 50 or 100  
22  $\mu$ g/ml. Samples were incubated at room temperature for 10 minutes and  
23 trypsin digestion stopped by addition of SDS-sample buffer. Samples were  
24 subjected to polyacrylamide gel electrophoresis and autoradiographed  
25 according to standard procedures.

26 Both the agonist and antagonist led to distinct patterns of trypsin  
27 sensitivity that were different from the result obtained by digestion of the  
28 unliganded receptor. Autoradiographic results indicated that trypsin  
29 concentrations of 25, 50 and 100  $\mu$ g/ml completely digested the radiolabeled

1 RAR- $\alpha$  in 10 minutes at room temperature in the absence of added retinoid.  
2 Prebinding of ATRA led to the appearance of two major protease resistant  
3 species. Prebinding of the RAR- $\alpha$ -selective antagonist AGN 193583 gave rise  
4 to a protease resistant species that was of lower molecular weight than that  
5 resulting from ATRA prebinding. This result demonstrated that a retinoid  
6 agonist and antagonist led to conformational changes detectable by virtue of  
7 altered trypsin sensitivities. Surprisingly, prebinding of AGN 193109 gave rise  
8 to a protease protection pattern that was indistinguishable from that  
9 produced by prebinding of ATRA.

10 The results presented above confirmed that AGN 193109 bound the  
11 RAR- $\alpha$  and altered its conformation. Interestingly, the nature of this  
12 conformational change more closely resembled that which resulted from  
13 binding of an agonist (ATRA) than the alteration produced by antagonist  
14 (AGN 193583) binding. Clearly, the mechanism of AGN 193109 dependent  
15 antagonism was unique.

16 We considered possible mechanisms that could account for the  
17 antagonist activity of AGN 193109. In particular, we used a standard gel shift  
18 assay to test whether AGN 193109 perturbed RAR/RXR heterodimer  
19 formation or inhibited the interaction between RAR and its cognate DNA  
20 binding site.

21 Example 9 describes a gel electrophoretic mobility-shift assay used to  
22 demonstrate that AGN 193109 neither inhibited RAR/RXR dimerization nor  
23 inhibited binding of dimers to a target DNA.

#### 24 Example 9

##### 25 Gel Shift Analysis

26 *In vitro* translated RAR- $\alpha$  was produced essentially as described under  
27 Example 8, except that <sup>35</sup>S-labeled methoinine was omitted. *In vitro*  
28 translated RXR- $\alpha$  was similarly produced using a pBluescript(II)(SK)-based  
29 vector containing the RXR- $\alpha$  cDNA described by Mangelsdorf, et al. in

1 *Nature* 345:224-229 (1990) as the template for generating *in vitro* transcripts.  
2 The labeled RAR- $\alpha$  and RXR- $\alpha$ , alone or in combination, or prebound with  
3 AGN 193109 ( $10^{-6}$  M) either alone or in combination, were allowed to  
4 interact with an end-labeled DR-5 RARE double-stranded probe having the  
5 sequence 5'-TCAGGTCACCAGGAGGTCAGA-3' (SEQ ID NO:1). The  
6 binding mixture was electrophoresed on a non-denaturing polyacrylamide gel  
7 and autoradiographed according to standard laboratory procedures. A single  
8 retarded species appearing on the autoradiograph that was common to all the  
9 lanes on the gel represented an undefined probe-binding factor present in the  
10 reticulocyte lysate. Only the RAR/RXR combination gave rise to a retinoid  
11 receptor-specific retarded species. Neither RAR alone nor RXR alone  
12 bound the probe to produce this shifted species. The presence of AGN  
13 193109 did not diminish this interaction.

14 These results indicated that AGN 193109 did not substantially alter  
15 either the homo- or hetero-dimerization properties of RAR- $\alpha$ . Further,  
16 AGN 193109 did not inhibit the interaction of receptor dimers with a DNA  
17 segment containing the cognate-binding site.

18 In view of the unique properties which characterized AGN 193109, we  
19 proceeded to investigate whether this antagonist could additionally inhibit the  
20 activity of unliganded RARs. The receptor/reporter system used to make this  
21 determination advantageously exhibited high level constitutive activity in the  
22 absence of added retinoid agonist. More specifically, these procedures  
23 employed the ER-RAR chimeric receptor and ERE-tk-Luc reporter system.  
24 The ERE-tk-Luc plasmid includes the region -397 to -87 of the estrogen  
25 responsive 5'-flanking region of the *Xenopus* vitellogenin A2 gene, described  
26 by Klein-Hitpass, et al. in *Cell* 46:1053-1061 (1986), ligated upstream of the  
27 HSV thymidine kinase promoter and luciferase reporter gene of plasmid tk-  
28 Luc. The ER-RAR chimeric receptors consisted of the estrogen receptor  
29 DNA binding domain fused to the "D-E-F" domain of the RARs. Those



1 having ordinary skill in the art appreciate this "D-E-F" domain functions to  
2 bind retinoid, to provide a retinoid inducible transactivation function and to  
3 provide a contact site for heterodimerization with RXR. Thus, luciferase  
4 expression in this reporter system was dependent on activation of the  
5 transfected chimeric receptor construct.

6 Example 10 describes the method used to demonstrate that AGN  
7 193109 inhibited basal gene activity attributable to unliganded RARs. These  
8 procedures were performed in the absence of added retinoid agonist. The  
9 results presented below provided the first indication that AGN 193109  
10 exhibited negative hormone activity.

#### 11 Example 10

##### 12 Repression of Basal Gene Activity of a Retinoid-Regulated 13 Reporter in Transiently Cotransfected Cell Lines

14 CV-1 cells were co-transfected with the ERE-tk-Luc reporter plasmid  
15 and either ER-RAR- $\alpha$ , ER-RAR- $\beta$  or ER-RAR- $\gamma$  expression plasmids. The  
16 ERE-tk-Luc plasmid contained the estrogen-responsive promoter element of  
17 the *Xenopus laevis* vitellogenin A2 gene and was substantially identical to the  
18 reporter plasmid described by Klein-Hitpass et al. in *Cell* 46:1053 (1986),  
19 except that the CAT reporter gene was substituted by a polynucleotide  
20 sequence encoding luciferase. The ER-RAR- $\alpha$ , ER-RAR- $\beta$  and ER-RAR- $\gamma$   
21 chimeric receptor-encoding polynucleotides employed in the co-transfection  
22 have been described by Graupner et al. in *Biochem. Biophys. Res. Comm.*  
23 179:1554 (1991). These polynucleotides were ligated into the pECE  
24 expression vector described by Ellis et al. in *Cell* 45:721 (1986) and expressed  
25 under transcriptional control of the SV-40 promoter. Calcium phosphate  
26 transfections were carried out exactly as described in Example 6 using 0.5  
27  $\mu\text{g}/\text{well}$  of reporter plasmid and either 0.05  $\mu\text{g}$ , 0.10  $\mu\text{g}$  or 0.2  $\mu\text{g}/\text{well}$  of  
28 receptor plasmid. Cells were dosed with vehicle alone (ethanol), ATRA ( $10^{-9}$   
29 to  $10^{-6}$  M), or AGN 193109 ( $10^{-9}$  to  $10^{-6}$  M) for 18 hours. Cell lysates and

1 luciferase activity measurements were performed as described in Example 6.

2 The results presented in Figures 3A, 4A and 5A confirmed that ATRA  
3 strongly induced luciferase expression in all transfectants. Basal level  
4 expression of luciferase for the three transfected chimeric RAR isoforms  
5 ranged from approximately 7,000 to 40,000 relative light units (rlu) and was  
6 somewhat dependent on the amount of receptor plasmid used in the  
7 transfection. Thus, the three chimeric receptors were activatable by ATRA,  
8 as expected. More specifically, all three receptors bound ATRA and  
9 activated transcription of the luciferase reporter gene harbored on the ERE-  
10 tk-Luc plasmid.

11 Figures 3B, 4B and 5B present AGN 193109 dose response curves  
12 obtained in the absence of any exogenous retinoid agonist. Interestingly,  
13 ER-RAR- $\alpha$  (Figure 3B) was substantially unaffected by AGN 193109, while  
14 the ER-RAR- $\beta$  and ER-RAR- $\gamma$  chimeric receptors (Figures 4B and 5B,  
15 respectively) exhibited an AGN 193109 dose responsive decrease in luciferase  
16 reporter activity.

17 We further investigated the negative hormone activity of AGN 193109  
18 by testing its ability to repress gene expression mediated by a chimeric RAR-  
19  $\gamma$  receptor engineered to possess a constitutive transcription activator  
20 domain. More specifically, we used a constitutively active RAR- $\gamma$  chimeric  
21 receptor fused to the acidic activator domain of HSV VP-16, called RAR- $\gamma$ -  
22 VP-16, in two types of luciferase reporter systems. The first consisted of the  
23 ERE-tk-Luc reporter cotransfected with ER-RARs and ER-RXR- $\alpha$ . The  
24 second utilized the  $\Delta$ MTV-TREp-Luc reporter instead of the ERE-tk-Luc  
25 reporter.

26 Example 11 describes the method used to demonstrate that AGN  
27 193109 could suppress the activity of a transcription activator domain of an  
28 RAR. The results presented below proved that AGN 193109 could suppress  
29 RAR-dependent gene expression in the absence of an agonist and confirmed

1 that AGN 193109 exhibited negative hormone activity.

## 2 Example 11

### 3 Repression of RAR-VP-16 Activity in Transiently

#### 4 Transfected Cells

5 CV-1 cells were transiently cotransfected according to the calcium  
6 phosphate coprecipitation technique described under Example 6 using 0.5  
7  $\mu\text{g}/\text{well}$  of the ERE-tk-Luc luciferase reporter plasmid, 0.1  $\mu\text{g}/\text{well}$  of the ER-  
8 RXR- $\alpha$  chimeric reporter expression plasmid, and either 0  $\mu\text{g}$  or 0.1  $\mu\text{g}/\text{well}$   
9 of the RAR- $\gamma$ -VP-16 expression plasmid. The chimeric receptor ER-RXR- $\alpha$   
10 consisted of the hormone binding domain (amino acids 181 to 458) of RXR-  
11  $\alpha$  (Mangelsdorf, et al. *Nature* 345:224-229 (1990)) fused to the estrogen  
12 receptor DNA binding domain (Graupner, et al. *Biochem. Biophys. Res.*  
13 *Comm.* 179:1554 (1991)) and was expressed from the SV-40 based expression  
14 vector pECE described by Ellis, et al. in *Cell* 45:721 (1986). RAR- $\gamma$ -VP-16 is  
15 identical to the VP16RAR- $\gamma$ 1 expression plasmid described by Nagpal et al.  
16 in *EMBO J.* 12:2349 (1993), the disclosure of which is hereby incorporated by  
17 reference, and encodes a chimeric protein having the activation domain of  
18 the VP-16 protein of HSV fused to the amino-terminus of full length RAR- $\gamma$ .  
19 At eighteen hours post-transfection, cells were rinsed with phosphate  
20 buffered saline (PBS) and fed with DMEM (Gibco-BRL) containing 10%  
21 FBS (Gemini Bio-Products) that had been extracted with charcoal to remove  
22 retinoids. Cells were dosed with an appropriate dilution of AGN 193109 or  
23 ATRA in ethanol vehicle or ethanol alone for 18 hours, then rinsed with PBS  
24 and lysed using 0.1 M  $\text{KPO}_4$  (pH 7.8), 1.0% TRITON X-100, 1.0 mM DTT, 2  
25 mM EDTA. Luciferase activity was measured according to the method  
26 described by de Wet, et al. in *Mol. Cell. Biol.* 7:725 (1987), using firefly  
27 luciferin (Analytical Luminescence Laboratory) and an EG&G Berthold 96-  
28 well plate luminometer. Luciferase values represented the mean  $\pm$  SEM of  
29 triplicate determinations.

1 As shown in Figure 6, CV-1 cells transfected with the ERE-tk-Luc  
2 reporter construct and the ER-RAR- $\alpha$  chimeric expression plasmid exhibited  
3 a weak activation of luciferase activity by ATRA, likely due to isomerization  
4 of ATRA to 9C-RA, the natural ligand for the RXRs (Heyman et al. *Cell*  
5 68:397 (1992)). Cells transfected with the same mixture of reporter and  
6 chimeric receptor plasmids but treated with AGN 193109 did not exhibit any  
7 effect on luciferase activity. As AGN 193109 does not bind to the RXRs,  
8 this latter result was expected. CV-1 cells similarly transfected with the ERE-  
9 tk-Luc reporter but with substitution of an ER-RAR chimeric receptor  
10 expression plasmid for ER-RXR- $\alpha$  exhibited a robust induction of luciferase  
11 activity following ATRA treatment.

12 In contrast, inclusion of the RAR- $\gamma$ -VP-16 expression plasmid with the  
13 ER-RXR- $\alpha$  and ERE-tk-Luc plasmids in the transfection mixture resulted in  
14 a significant increase in the basal luciferase activity as measured in the  
15 absence of any added retinoid. This increase in basal luciferase activity  
16 observed for the ER-RXR- $\alpha$ /RAR- $\gamma$ -VP-16 cotransfectants, when compared  
17 to the result obtained using cells transfected with ER-RXR- $\alpha$  alone, indicated  
18 that recombinant ER-RXR- $\alpha$  and RAR- $\gamma$ -VP-16 proteins could  
19 heterodimerize. Interaction of the heterodimer with the cis-regulatory  
20 estrogen responsive element led to a targeting of the VP-16 activation  
21 domain to the promoter region of the ERE-tk-Luc reporter. Treatment of  
22 such triply transfected cells with ATRA led to a modest increase of luciferase  
23 activity over the high basal level. However, treatment of the triple  
24 transfectants with AGN 193109 resulted in a dose dependent decrease in  
25 luciferase activity. Importantly, Figure 6 shows that AGN 193109 treatment  
26 of cells cotransfected with ER-RXR- $\alpha$  and RAR- $\gamma$ -VP-16 led to repression  
27 of luciferase activity with maximal inhibition occurring at approximately  $10^{-8}$   
28 M AGN 193109.

29 Our observation that AGN 193109 repressed the constitutive

1 transcriptional activation function of RAR- $\gamma$ -VP-16 in the presence of an  
2 RXR was explained by a model wherein binding of AGN 193109 to the RAR  
3 induced a conformational change in the RAR which stabilizes a negative  
4 conformation that facilitates the binding of a trans-acting negative coactivator  
5 protein. When the AGN 193109/RAR complex is bound by the NCP, the  
6 RAR is incapable of upregulating transcription of genes that are ordinarily  
7 responsive to activated RARs. Our model further proposes that the  
8 intracellular reservoir of NCP is in limiting concentration in certain contexts  
9 and can be depleted by virtue of AGN 193109 stimulated complexation with  
10 RARs.

11 The results presented in Figure 6 additionally indicated that even at  $10^{-6}$   
12 M AGN 193109, the ER-RXR- $\alpha$  and RAR- $\gamma$ -VP-16 proteins could interact  
13 to form heterodimers competent for activating transcription of the reporter  
14 gene. More specifically, cells transfected with ER-RXR- $\alpha$  and RAR- $\gamma$ -VP-16  
15 and treated with AGN 193109 at a concentration ( $10^{-8}$ - $10^{-6}$  M) sufficient to  
16 provide maximal inhibition, gave luciferase activity readings of approximately  
17 16,000 rlu. Conversely, cells transfected only with ER-RXR- $\alpha$  and then  
18 treated with AGN 193109 at a concentration as high as  $10^{-6}$  M exhibited  
19 luciferase expression levels of only approximately 8,000 rlu. The fact that a  
20 higher level of luciferase activity was obtained in cells that expressed both  
21 ER-RXR- $\alpha$  and RAR- $\gamma$ -VP-16, even in the presence of  $10^{-6}$  M AGN 193109  
22 demonstrated the persistence of an interaction between the two recombinant  
23 receptors. The repression of RAR- $\gamma$ -VP-16 activity by AGN 193109  
24 suggested that modulation of NCP interaction can be codominate with VP-16  
25 activation. Accordingly, we realized that it may be possible to modulate the  
26 expression of genes which are not ordinarily regulated by retinoids in an  
27 AGN 193109 dependent manner.

28 Candidates for AGN 193109 regulatable genes include those that are  
29 activated by transcription factor complexes which consist of non-RAR factors

1 that associate or heterodimerize with RARs, wherein the non-RAR factor  
2 does not require an RAR agonist for activation. While stimulation with an  
3 RAR agonist may have substantially no effect on the expression of such  
4 genes, administration with AGN 193109 can promote formation of inactive  
5 transcription complexes comprising AGN 193109/RAR/NCP. Consequently,  
6 addition of the AGN 193109 retinoid negative hormone can down-regulate  
7 transcription of an otherwise retinoid-insensitive gene.

8 This same mechanism can account for the observation that AGN  
9 193109 can repress the activity of the tissue transglutaminase (TGase) gene in  
10 HL-60 cells. A retinoid response element consisting of three canonical  
11 retinoid half sites spaced by 5 and 7 base pairs has been identified in the  
12 transcription control region of this gene. While TGase can be induced by  
13 RXR-selective agonists, it is not responsive to RAR-selective agonists. The  
14 TGase retinoid response element is bound by an RAR/RXR heterodimer  
15 (Davies et al. in Press). Interestingly, AGN 193109 is able to repress TGase  
16 activity induced by RXR agonists. This AGN 193109 mediated repression  
17 can be accounted for by the ability of this negative hormone to sequester  
18 NCPs to the RAR component of the heterodimer, thereby repressing the  
19 activity of the associated RXR.

20 We have also obtained results which support conclusions identical to  
21 those presented under Example 11 by employing RAR- $\gamma$ -VP-16 and  
22 expression constructs and the  $\Delta$ MTV-TREp-Luc reporter plasmid instead of  
23 the RAR- $\gamma$ -VP-16 and ER-RXR- $\alpha$  expression constructs in combination with  
24 the ERE-tk-Luc reporter plasmid. Consistent with the results presented  
25 above, we found that RAR- $\gamma$ -VP-16 activity at the  $\Delta$ MTV-TREp-Luc  
26 reporter was inhibited by AGN 193109. Therefore, AGN 193109 repressed  
27 RAR- $\gamma$ -VP-16 activity when this chimeric receptor was directly bound to a  
28 retinoic acid receptor response element instead of indirectly bound to an  
29 estrogen response element in the promoter region of the reporter plasmid.

1 These findings demonstrated that an assay for identifying agents having  
2 negative hormone activity need not be limited by the use of a particular  
3 reporter plasmid. Instead, the critical feature embodied by an experimental  
4 system useful for identifying retinoid negative hormones involves detecting  
5 the ability of a compound to repress the activity of an RAR engineered to  
6 contain a constitutive transcription activation domain.

7 Generally, retinoid negative hormones can be identified as the subset  
8 of retinoid compounds that repress within a transfected cell the basal level  
9 expression of a reporter gene that is transcriptionally responsive to direct or  
10 indirect binding by a retinoid receptor or a chimeric receptor that includes at  
11 least the domains of the retinoid receptor located C-terminal to the DNA  
12 binding domain of that receptor. This approach has been adapted to a  
13 screening method useful for identifying retinoid negative hormones. In the  
14 various embodiments of the invented screening method, the structure of the  
15 receptor for which a negative hormone is sought is variable. More  
16 specifically, the retinoid receptor can be either of the RAR or the RXR  
17 subtype. The receptor can optionally be engineered to include a constitutive  
18 transcription activator domain. The retinoid receptor used to screen for  
19 negative hormones optionally contains a heterologous DNA binding domain  
20 as a substitute for the DNA binding domain endogenous to the native  
21 receptor. However, when a second receptor is used in the screening method,  
22 and where the second receptor can dimerize with the retinoid receptor for  
23 which a negative hormone is sought, then that retinoid receptor may not  
24 require a DNA binding domain because it can be linked to the transcription  
25 control region of the reporter gene indirectly through dimerization with the  
26 second receptor which is itself bound to the transcription control region.  
27 In the practice of the screening method, the ability of a compound to  
28 repress the basal expression of a reporter is typically measured in an *in vitro*  
29 assay. Basal expression represents the baseline level of reporter expression in

1 transfected cells under conditions where no exogenously added retinoid  
2 agonist is present. Optionally, steps may be taken to remove endogenous  
3 retinoid ligands from the environment of the transfected cells via procedures  
4 such as charcoal extraction of the serum that is used to culture cells *in vitro*.

5       Examples of reporter genes useful in connection with the screening  
6 method include those encoding luciferase, beta galactosidase,  
7 chloramphenicol acetyl transferase or cell surface antigens that can be  
8 detected by immunochemical means. In practice, the nature of the reporter  
9 gene is not expected to be critical for the operability of the method.  
10 However, the transcriptional regulatory region of the reporter construct must  
11 include one or more cis-regulatory elements that are targets of transcription  
12 factors for which negative hormones are being sought. For example, if one  
13 desires to identify RAR negative hormones, then the transcriptional  
14 regulatory region of the reporter construct could contain a cis-regulatory  
15 element that can be bound by an RAR-containing protein. In this example,  
16 there should be correspondence between the DNA binding domain of the  
17 RAR and the cis-regulatory element of the transcriptional regulatory region  
18 of the reporter construct. Thus, if a chimeric RAR having a constitutive  
19 transcription activator domain and a DNA binding domain that can bind cis-  
20 regulatory estrogen responsive elements is employed in the screening method,  
21 then the transcriptional regulatory region of the reporter construct should  
22 contain an estrogen responsive element.

23       Examples of cis-regulatory elements that directly bind retinoid  
24 receptors (RAREs) useful in connection with the reporter assay are disclosed  
25 by Mangelsdorf et al. in *The Retinoid Receptors* in The Retinoids: Biology,  
26 Chemistry and Medicine, 2nd edition, eds. Sporn et al., Raven Press, Ltd.,  
27 New York (1994), the disclosure of which has been incorporated by reference  
28 hereinabove. Examples of cis-regulatory elements that indirectly bind  
29 chimeric receptors include DNA binding sites for any DNA binding protein



1 for which the DNA binding domain of the protein can be incorporated into a  
2 chimeric receptor consisting of this DNA binding domain attached to a  
3 retinoid receptor. Specific examples of heterologous DNA binding domains  
4 that can be engineered into chimeric receptors and that will recognize  
5 heterologous cis-regulatory elements include those recognizing estrogen  
6 responsive elements. Thus, the retinoid receptor portion of a chimeric  
7 receptor useful in connection with the screening method need not contain the  
8 DNA binding of the retinoid receptor but must contain at least the ligand  
9 binding domain of the retinoid receptor.

10 A further example of indirect retinoid receptor binding to the cis-  
11 regulatory element includes the use of a protein that can bind the cis-  
12 regulatory element and dimerize with a retinoid receptor. In this case, the  
13 retinoid receptor associates with the cis-regulatory element only by  
14 association with the protein responsible for DNA binding. An example of  
15 such a system would include the use of a fusion protein consisting of a  
16 heterologous DNA binding domain fused to an RXR, containing at least the  
17 domain of the RXR responsible for dimerization with RARs. Cointroduced  
18 RARs can dimerize with such a fusion protein bound to the cis-regulatory  
19 element. We anticipate that any cis-regulatory element-binding protein that  
20 dimerizes with RARs to result in an indirect association of the RAR with the  
21 cis-regulatory element will also be suitable for use with the negative hormone  
22 screening method.

23 In a preferred embodiment of the screening method, retinoid negative  
24 hormones are identified as those retinoids that repress basal expression of an  
25 engineered RAR transcription factor having increased basal activity.  
26 Although not essential for operability of the screening method, the  
27 engineered RAR employed in the following Example included a constitutive  
28 transcription activating domain. Use of this chimeric receptor advantageously  
29 provided a means by which the basal expression of a reporter gene could be

1 elevated in the absence of any retinoid. Although we have employed  
2 transient transfection in the procedures detailed above, stably transfected cell  
3 lines constitutively expressing the chimeric receptor would also be useful in  
4 connection with the screening method.

5 As disclosed in the following Example, a chimeric retinoid receptor  
6 having a constitutive transcription activator domain was heterodimerizable  
7 with a second receptor engineered to contain a DNA binding domain specific  
8 for an estrogen responsive cis-regulatory element. In this case the chimeric  
9 retinoid receptor having a constitutive transcription activator domain  
10 associates with the cis-regulatory region controlling reporter gene expression  
11 indirectly via binding to a second receptor that binds a DNA target sequence.  
12 More particularly, the second receptor was engineered to contain a DNA  
13 binding domain that recognized an estrogen responsive element.  
14 Advantageously, the reporter gene having an estrogen responsive element in  
15 the upstream promoter region was unresponsive to retinoid agonists in the  
16 absence of the transfected chimeric receptor having the constitutive  
17 transcription activator domain. Accordingly, all reporter gene activity was  
18 attributed to the transfected receptors. The combination use of the estrogen  
19 responsive element DNA binding domain and the estrogen responsive  
20 element cis-regulatory element are intended to be illustrative only. Those  
21 having ordinary skill in the art will realize that other combinations of  
22 engineered receptors having specificity for non-RARE cis-regulatory elements  
23 will also be useful in the practice of the invented screening method.

24 Cells useful in connection with the screening method will be eukaryotic  
25 cells that can be transfected. The cells may be animal cells such as human,  
26 primate or rodent cells. We have achieved very good results using CV-1  
27 cells, but reasonably expect that other cultured cell lines could also be used  
28 successfully. Any of a number of conventional transfection methods known  
29 in the art can be used to introduce an expression construct encoding the

1 chimeric retinoid receptor having a constitutive transcription activator  
2 domain.

3 The constitutive transcription activator domain will consist of a  
4 plurality of amino acids which will likely have an overall acidic character as  
5 represented by a negative charge under neutral pH conditions. For example,  
6 the constitutive transcription activator domain may have an amino acid  
7 sequence which is also found in viral transcription factors. One example of a  
8 viral transcription factor having a constitutive transcription activator domain  
9 is the herpes simplex virus 16. However, other viral or synthetic transcription  
10 activator domains would also be useful in the construction of expression  
11 constructs encoding the chimeric retinoid receptor having a constitutive  
12 transcription activator domain.

13 As described below, we have developed a generalized screening  
14 method useful for identifying retinoid negative hormones. This screening  
15 method provides a means for distinguishing simple antagonists from negative  
16 hormones. Table 12 lists several retinoid compounds which exhibit potent  
17 affinity for RAR- $\gamma$  yet, with the exception of ATRA, did not transactivate  
18 this receptor in a transient cotransfection transactivation assay. We therefore  
19 tested these compounds to determine which were RAR- $\gamma$  antagonists and  
20 which, if any, of these antagonists exhibited negative hormone activity.

21 Example 12 describes the method used to identify retinoid compounds  
22 that were antagonists, and the subset of antagonists that exhibited negative  
23 hormone activity.

#### 24 Example 12

##### 25 Assay for Retinoid Negative Hormones

26  $4 \times 10^4$  CV-1 cells were transfected by the calcium phosphate  
27 coprecipitation procedure described in *Molecular Cloning: A Laboratory*  
28 *Manual* (Sambrook et al. eds. Cold Spring Harbor Lab Publ. 1989) using 0.5  
29  $\mu\text{g}$  ERE-tk-Luc reporter plasmid and 0.1  $\mu\text{g}$  ER-RAR- $\gamma$  (Graupner et al.

1 *Biochem. Biophys. Res. Comm.* 179:1554 (1991)) chimeric expression plasmid.  
 2 After 18 hours, cells were rinsed with PBS and fed with DMEM (Gibco-  
 3 BRL) containing 10% activated charcoal extracted FBS (Gemini Bio-  
 4 Products). Cells were treated with  $10^{-8}$  M ATRA in ethanol or ethanol alone.  
 5 In addition, ATRA treated cells were treated with  $10^{-9}$ ,  $10^{-8}$ ,  $10^{-7}$  or  $10^{-6}$  M of  
 6 the compounds listed in Table 12. After 18 hours, cells were rinsed in PBS  
 7 and lysed in 0.1 M  $\text{KPO}_4$  (pH 7.8), 1.0% TRITON X-100, 1.0 mM DTT, 2  
 8 mM EDTA. Luciferase activities were measured as described by deWet et al.  
 9 in *Mol. Cell. Biol.* 7:725 (1987).

TABLE 12

11	Compound	Kd (nM) @ RAR- $\gamma^a$	EC <sub>50</sub> (nM) @ RAR- $\gamma^b$
12	ATRA	12	17
13	AGN 193109	6	na
14	(Compound 60)		
15	AGN 193174	52	na
16	(Compound 34a)		
17	AGN 193199	30	na
18	AGN 193385	25	na
19	(Compound 23)		
20	AGN 193389	13	na
21	(Compound 25)		
22	AGN 193840	40	na
23	AGN 193871	30	na
24	(Compound 50)		

25  
 26 <sup>a</sup> Relative affinity (Kd) determined by competition of  $^3\text{H}$ -ATRA  
 27 binding to baculovirus expressed RAR- $\gamma$  and application of the Cheng-  
 28 Prusoff equation.

29 <sup>b</sup> EC<sub>50</sub> measured in CV-1 cells transiently cotransfected with  
 30  $\Delta\text{MTV-TREp-Luc}$  and RS-RAR- $\gamma$ . "na" denotes no activity.

1 As indicated by the results presented in part in Figure 7 and in Table  
2 12, with the exception of ATRA, all of the compounds listed in Table 12  
3 were retinoid antagonists at RAR- $\gamma$ .

4 The RAR- $\gamma$  antagonists identified in Table 12 were next screened to  
5 determine which, if any, were also retinoid negative hormones.  $4 \times 10^4$  CV-1  
6 cells were transfected according to the calcium phosphate procedure  
7 described in *Molecular Cloning: A Laboratory Manual* (Sambrook et al. eds.  
8 Cold Spring Harbor Lab Publ. 1989) using 0.5  $\mu$ g ERE-tk-Luc reporter  
9 plasmid and 0.1  $\mu$ g ER-RXR- $\alpha$  (Graupner et al. *Biochem. Biophys. Res.*  
10 *Comm.* 179:1554 (1991)) and 0.2  $\mu$ g RAR- $\gamma$ -VP-16 (Nagpal et al. *EMBO J.*  
11 12:2349 (1993)) chimeric expression plasmids. After 18 hours, cells were  
12 rinsed with PBS and fed with DMEM (Gibco-BRL) containing 10% activated  
13 charcoal extracted FBS (Gemini Bio-Products). Cells were treated with  $10^{-9}$ ,  
14  $10^{-8}$ ,  $10^{-7}$  or  $10^{-6}$  M of each of the compounds listed in Table 12. Treatment  
15 with ethanol vehicle alone served as the negative control. After 18 hours,  
16 cells were rinsed in PBS and lysed in 0.1 M  $\text{KPO}_4$  (pH 7.8), 1.0% TRITON  
17 X-100, 1.0 mM DTT, 2 mM EDTA. Luciferase activities were measured as  
18 previously by deWet et al. in *Mol Cell. Biol.* 7:725 (1987).

19 As shown in Figure 8, the retinoid antagonists of Table 12 could be  
20 separated into two classes by virtue of their effect on the constitutive  
21 transcription activation function of the RAR- $\gamma$ -VP-16 chimeric retinoid  
22 receptor. One group, which included AGN 193174, AGN 193199 and AGN  
23 193840, did not repress RAR- $\gamma$ -VP-16 activity even though they were ATRA  
24 antagonists. In contrast AGN 193109, AGN 193385, AGN 193389 and AGN  
25 193871 exhibited a dose dependent repression of RAR- $\gamma$ -VP-16 constitutive  
26 activity. Therefore, while the compounds of both groups were RAR- $\gamma$   
27 antagonists, only those of the second group exhibited negative hormone  
28 activity. This assay advantageously distinguished retinoid negative hormones  
29 from simple retinoid antagonists.

1       The foregoing experimental results proved that AGN 193109 met the  
2 criteria that define a negative hormone. More specifically, the results  
3 presented under Example 11 demonstrated that AGN 193109 had the  
4 capacity to exert inhibitory activity at the RARs even in the absence of  
5 exogenously added retinoid ligands. As such, this compound possessed  
6 biological activities that did not depend upon blockade of the interaction  
7 between the RARs and agonists such as ATRA and AGN 191183. These  
8 findings led us to conclude that AGN 193109 stabilized interactions between  
9 RARs and NCPs. As diagrammed in Figure 9, NCP/RAR/PCP interactions  
10 exist in an equilibrium state. An agonist serves to increase PCP interactions  
11 and decrease NCP interactions, while an inverse agonist or negative hormone  
12 stabilizes NCP and decreases PCP interactions. As indicated previously, our  
13 experimental results suggested that the intracellular availability of NCP for  
14 other receptors can be modulated by AGN 193109 administration. More  
15 specifically, we discovered that AGN 193109 can promote complexation of  
16 NCP with RARs, thereby reducing the intracellular reservoir of NCP  
17 available for interaction with transcription factors other than the RARs.

18       We next examined the effect of AGN 193109 on agonist-mediated  
19 inhibition of AP-1 dependent gene expression. In *Endocr. Rev.* 14:651 (1993),  
20 Pfhal disclosed that retinoid agonists can down-regulate gene expression by a  
21 mechanism that involved inhibition of AP-1 activity. We postulated that  
22 AGN 193109 could have had either of two effects when used in combination  
23 with a retinoid agonist in a model system designed to measure AP-1 activity.  
24 First, AGN 193109 could conceivably have antagonized the effect of the  
25 agonist, thereby relieving the agonist-dependent inhibition of AP-1 activity.  
26 Alternatively, AGN 193109 could have potentiated the agonist's activity,  
27 thereby exaggerating the agonist-dependent inhibition of AP-1 activity.

28       Example 13 describes the methods used to demonstrate that AGN  
29 193109 potentiated the anti-AP-1 activity of a retinoid agonist. As disclosed

1 below, the AGN 191183 retinoid agonist weakly inhibited AP-1 dependent  
2 gene expression. The combination of AGN 193109 and the retinoid agonist  
3 strongly inhibited AP-1 dependent gene expression. By itself, AGN 193109  
4 had substantially no anti-AP-1 activity.

### 5 Example 13

#### 6 AGN 193109 Potentiates the Anti-AP-1 Activity

##### 7 of a Retinoid Agonist

8 HeLa cells were transfected with 1  $\mu$ g of the Str-AP1-CAT reporter  
9 gene construct and 0.2  $\mu$ g of plasmid pRS-hRAR $\alpha$ , described by Giguere et  
10 al. in *Nature* 33:624 (1987), using LIPOFECTAMINE (Life Technologies,  
11 Inc.). Str-AP1-CAT was prepared by cloning a DNA fragment corresponding  
12 to positions -84 to +1 of the rat stromelysin-1 promoter (Matrisian et al.,  
13 *Mol. Cell. Biol.* 6:1679 (1986)) between the HindIII-BamHI sites of  
14 pBLCAT3 (Luckow et al., *Nucl. Acids Res.* 15:5490 (1987)). This sequence of  
15 the stromelysin-1 promoter contains an AP1 motif as its sole enhancer  
16 element (Nicholson et al., *EMBO J.* 9:4443 (1990)). The promoter sequence  
17 was prepared by annealing two synthetic oligonucleotides having sequences:  
18 5'-

19 AGAAGCTTATGGAAGCAATTATGAGTCAGTTTGCGGGTGACTCTG  
20 CAAATACTGCCACTCTATAAAAGTTGGGCTCAGAAAGGTGGACCTC  
21 GAGGATCCAG-3'

22 (SEQ ID NO:2), and 5'-

23 CTGGATCCTCGAGGTCCACCTTTCTGAGCCCAACTTTTATAGAGTG  
24 GCAGTATTTGCAGAGTCACCCGCAAAGTACTGACTCATAATTGCTTCCA  
25 TAAGCTTCT-3' (SEQ ID NO:3). Procedures involving transfection,

26 treatment with appropriate compounds and measurement of CAT activity  
27 were carried out as described by Nagpal et al. in *J. Biol. Chem.* 270:923  
28 (1995), the disclosure of which is hereby incorporated by reference.

29 The results of these procedures indicated that AGN 193109

1 potentiated the anti-AP-1 activity of the retinoid agonist, AGN 191183.  
2 More specifically, in the concentration range of from  $10^{-12}$  to  $10^{-10}$  M, AGN  
3 191183 did not inhibit the TPA-induced Str-AP1-CAT expression. Treatment  
4 with AGN 193109 in the concentration range of from  $10^{-10}$  to  $10^{-8}$  M did not  
5 substantially inhibit AP-1 mediated reporter activity. However, the results  
6 presented in Figure 10 indicated that stimulation of the transfectants with the  
7 combination of AGN 193109 ( $10^{-8}$  M) and AGN 191183 in the concentration  
8 range of from  $10^{-12}$  to  $10^{-10}$  M substantially inhibited TPA-induced Str-AP1-  
9 CAT expression by an amount of from 12% to 21%. Therefore, AGN  
10 193109 potentiated the anti-AP-1 activity of AGN 191183 under conditions  
11 where this retinoid agonist ordinarily did not inhibit AP-1 activity.

12 We reasoned that AGN 193109 potentiated the agonist-mediated  
13 repression of AP-1 activity by a mechanism that likely involved AGN 193109-  
14 dependent sequestration of NCPs onto RARs. RARs belong to a  
15 superfamily of nuclear receptors that also includes receptors for 1,25-  
16 dihydroxyvitamin  $D_3$ , glucocorticoid, thyroid hormone, estrogen and  
17 progesterone. It was a reasonable assumption that the ability to bind NCPs  
18 may be shared among different members of the nuclear receptor superfamily.  
19 This led us to speculate that AGN 193109 could potentiate the anti-AP-1  
20 activity of one or more of the ligands that interact with this superfamily of  
21 nuclear receptors.

22 The results presented in the preceding Example clearly indicated that  
23 AGN 193109 potentiated the anti-AP-1 activity of a retinoid agonist. More  
24 specifically, AGN 193109 lowered the threshold dose at which the anti-AP-1  
25 activity of AGN 191183 could be detected. Since AGN 193109 has  
26 substantially no anti-AP-1 activity by itself, its effect on nuclear receptor  
27 agonists was synergistic. We also found that the AGN 193109 negative  
28 hormone potentiated the anti-AP-1 activity of 1,25-dihydroxyvitamin  $D_3$ , the  
29 natural ligand for the vitamin  $D_3$  receptor.



1       The observed synergy between AGN 193109 and AGN 191183 in the  
2 preceding Example necessarily implied that the anti-AP-1 activity of the  
3 retinoid agonist and the AGN 193109-mediated potentiation of that activity  
4 must result from different mechanisms. If the mechanisms of action of the  
5 two agents were identical, then it follows that the effectiveness of the  
6 combination of AGN 193109 and the agonist would have been additive.  
7 Instead, the combination was shown to be more effective than either agent  
8 alone, an effect that could not have been predicted in advance of this finding.

9       Significantly, the AGN 193109-mediated potentiation of the RAR  
10 agonist was performed using an approximately 100-fold molar excess of AGN  
11 193109 over that of the retinoid agonist. Accordingly, the majority of RARs  
12 should have been bound by AGN 193109 leaving very few RARs available for  
13 agonist binding. In spite of this fact, the population of RARs that were not  
14 bound by AGN 193109 were able to bind retinoid agonist and vigorously  
15 stimulate an agonist-dependent response measurable as an inhibition of  
16 reporter gene expression. Thus, our data suggested possible heterogeneity of  
17 RARs that are induced by AGN 193109.

18       The negative hormone activity of AGN 193109, attributed to its ability  
19 to promote the interaction of RARs and NCPs, provided a basis for  
20 understanding the synergy between AGN 193109 and retinoid agonists. Our  
21 results were fully consistent with a model in which AGN 193109 treatment of  
22 cells promoted binding of RARs and NCPs, thereby reducing the number of  
23 free NCP and free RAR within the cell. This results in the generation of two  
24 populations of RARs that are functionally distinct. The first population is  
25 represented by RARs associated with NCPs. Such AGN 193109/RAR/NCP  
26 complexes cannot be activated by retinoid agonists. The second population  
27 consists of RARs that are not bound by NCP, and that remain available for  
28 interaction with agonists. This latter population is designated "RAR\*" to  
29 indicate free RARs in an environment substantially depleted of NCP.

1       The RAR\*s have decreased probabilities of association with NCP  
2 through equilibrium binding and have an increased sensitivity to retinoid  
3 agonists measurable, for example, as anti-AP-1 activity. This is so because,  
4 while the intracellular reservoir of NCP is depleted by virtue of AGN 193109  
5 administration, the reservoir of PCP has not been depleted. Accordingly,  
6 free RAR\*s can bind a retinoid agonist and interact with PCP factors in an  
7 environment substantially depleted of NCP. The ability of AGN 193109 to  
8 increase the sensitivity of other nuclear receptors to their respective agonists  
9 can be attributed to the ability of these different nuclear receptors to interact  
10 with the same NCPs that interact with AGN 193109/RAR complexes. This  
11 model of AGN 193109-mediated modulation of NCP availability for nuclear  
12 receptor family members is schematically represented in Figure 11.

13       This mechanistic model led us to predict that AGN 193109 could  
14 modulate the activities of nuclear receptor ligands other than retinoid  
15 agonists. As illustrated in the following Example, we confirmed that AGN  
16 193109 potentiated the activity of 1,25-dihydroxyvitamin D<sub>3</sub> in an *in vitro*  
17 transactivation assay.

18       Example 14 describes the methods used to demonstrate that AGN  
19 193109 enhanced the activity of 1,25-dihydroxyvitamin D<sub>3</sub> in a transactivation  
20 assay.

#### 21                               Example 14

##### 22               AGN 193109 Potentiates 1,25-Dihydroxyvitamin D<sub>3</sub> Activity

23       Hela cells were transfected using the cationic liposome-mediated  
24 transfection procedure described by Felgner et al. in *Proc. Natl. Acad. Sci.*  
25 *USA* 84:7413 (1987). 5 X 10<sup>4</sup> cells were plated in 12-well multiwell plates and  
26 grown in DMEM supplemented with 10% FBS. Cells were cotransfected in  
27 serum-free medium using 2 µg/well of LIPOFECTAMINE reagent (Life  
28 Technologies, Inc.) with 0.7 µg of the reporter plasmid MTV-VDRE-Luc,  
29 containing two copies of the 1,25-dihydroxyvitamin D<sub>3</sub> response element

1 5'-GTACAAGGTTTCACGAGGTTTCACGTCTTA-3' (SEQ ID NO:4) from  
2 the mouse osteopontin gene (Ferrara et al. *J. Biol. Chem.* 269:2971 (1994))  
3 ligated into the reporter plasmid  $\Delta$ MTV-Luc (Heyman et al. in *Cell* 68:397  
4 (1992)), and 0.3  $\mu$ g of the plasmid pGEM3Z (Pharmacia, Inc.) as carrier  
5 DNA to bring the final concentration of DNA to 1.0  $\mu$ g per well. After six  
6 hours of transfection, cells were fed with growth medium containing charcoal  
7 extracted FBS at a final concentration of 10%. Eighteen hours after  
8 transfection cells were treated with vehicle alone (ethanol) or AGN 193109 in  
9 ethanol at a final concentration of either  $10^{-8}$  or  $10^{-7}$  M. Six hours later 1,25-  
10 dihydroxyvitamin D<sub>3</sub> was added in ethanol to a final concentration of from  $10^{-10}$   
11 to  $10^{-7}$  M. Cells were lysed and harvested eighteen hours following 1,25-  
12 dihydroxyvitamin D<sub>3</sub> treatment. Luciferase activity was measured as described  
13 above. This experimental system allowed a convenient method of monitoring  
14 and quantitating 1,25-dihydroxyvitamin D<sub>3</sub>-dependent gene expression.

15 The results presented in Figure 12 indicated that, when compared with  
16 the result obtained using 1,25-dihydroxyvitamin D<sub>3</sub> alone, AGN 193109  
17 coadministered with 1,25-dihydroxyvitamin D<sub>3</sub> shifted the dose response curve  
18 to the left. This confirmed that AGN 193109 potentiated the effectiveness of  
19 1,25-dihydroxyvitamin D<sub>3</sub> in the *in vitro* transactivation assay. More  
20 specifically, Figure 12 graphically illustrates that an AGN 193109  
21 concentration as low as 10 - 100 nM rendered the 1,25-dihydroxyvitamin D<sub>3</sub>  
22 approximately 10 fold more active. While a 1,25-dihydroxyvitamin D<sub>3</sub>  
23 concentration of  $10^{-8}$  M was required to produce a luciferase expression of  
24 approximately 2,000 rlu, only one-tenth as much 1,25-dihydroxyvitamin D<sub>3</sub>  
25 was required to produce the same luciferase output when the vitamin was  
26 coadministered with AGN 193109 at a concentration of  $10^{-8}$  -  $10^{-7}$  M.  
27 Although not shown on the graph in Figure 12, substantially identical results  
28 were obtained using AGN 193109 concentrations of  $10^{-9}$  M and  $10^{-8}$  M. Thus,  
29 coadministration with AGN 193109 substantially reduced the amount of 1,25-

1 dihydroxyvitamin D<sub>3</sub> that was required to produce a similar effect in the  
2 absence of the negative hormone.

3 Interestingly, when the above procedure was repeated with  
4 cotransfection of a vitamin D receptor (VDR) expression plasmid, there was  
5 a coincident decrease in the ability of AGN 193109 to potentiate the activity  
6 of 1,25-dihydroxyvitamin D<sub>3</sub>. We interpreted this result as indicating that  
7 over-expression of VDRs could affect the ability of AGN 193109 to  
8 potentiate the activity of 1,25-dihydroxyvitamin D<sub>3</sub>. Thus, the intracellular  
9 concentration of a ligand receptor, which may differ in a tissue-specific  
10 fashion, can influence the ability of AGN 193109 to potentiate the activity of  
11 a ligand that binds the receptor. This was again consistent with a model in  
12 which titratable NCPs contributed to the regulation of the Vitamin D<sub>3</sub>  
13 response, and supported the model set forth above.

14 As illustrated in the following Example, we also confirmed that AGN  
15 193109 potentiated the anti-AP-1 activity of 1,25-dihydroxyvitamin D<sub>3</sub>. Our  
16 model for the activity of AGN 193109 action explains this observation by  
17 invoking that NCPs avidly-associate with RARs in the presence of this drug.  
18 Endogenous vitamin D receptors present in HeLa cells likely were rendered  
19 more sensitive to the 1,25-dihydroxyvitamin D<sub>3</sub> ligand, with the consequence  
20 of exaggerating the ability of this ligand to inhibit expression from the Str-  
21 AP1-CAT reporter.

22 Example 15 describes the methods used to demonstrate that AGN  
23 193109 potentiated the anti-AP-1 activity of 1,25-dihydroxyvitamin D<sub>3</sub>.

#### 24 Example 15

#### 25 AGN 193109 Potentiates the Anti-AP-1 Activity 26 of 1,25-Dihydroxyvitamin D<sub>3</sub>

27 HeLa cells were transfected with 1 µg of Str-AP1-CAT using  
28 LIPOFECTAMINE according to the method described by Nagpal et al. in *J.*  
29 *Biol. Chem.* 270:923 (1995). Transfected cells were treated with AGN 193109

1 alone ( $10^{-9}$  to  $10^{-7}$  M), 1,25-dihydroxyvitamin D<sub>3</sub> alone ( $10^{-12}$  to  $10^{-7}$  M) or  
2 1,25-dihydroxyvitamin D<sub>3</sub> ( $10^{-12}$  to  $10^{-7}$  M) in the presence of  $10^{-8}$  M AGN  
3 193109.

4 The results of these procedures indicated that AGN 193109  
5 potentiated the ability of 1,25-dihydroxyvitamin D<sub>3</sub> to inhibit TPA-induced  
6 AP-1 activity. When used alone in the concentration range of from  $10^{-9}$  to  
7  $10^{-7}$  M, AGN 193109 had no detectable anti-AP-1 activity. The results  
8 presented in Figure 13 indicated that 1,25-dihydroxyvitamin D<sub>3</sub> repressed  
9 TPA-stimulated activity only in the  $10^{-8}$  and  $10^{-7}$  M concentration range.  
10 Analysis of 1,25-dihydroxyvitamin D<sub>3</sub> mediated repression of TPA stimulated  
11 CAT activity in the presence of  $10^{-8}$  M AGN 193109 indicated that anti-AP-1  
12 activity was detectable at  $10^{-10}$  and  $10^{-9}$  M 1,25-dihydroxyvitamin D<sub>3</sub> and an  
13 increase in activity at  $10^{-8}$  and  $10^{-7}$  M doses compared to 1,25-  
14 dihydroxyvitamin D<sub>3</sub> treatment alone. This AGN 193109 dependent  
15 modulation of 1,25-dihydroxyvitamin D<sub>3</sub> mediated anti-AP-1 activity was  
16 consistent with our model in which NCP sequestration to RARs made the  
17 NCP unavailable for interaction with other nuclear receptor family members.  
18 Accordingly, the receptors were rendered more sensitive to the 1,25-  
19 dihydroxyvitamin D<sub>3</sub> treatment.

20 The mechanisms underlying RAR mediated transactivation and anti-  
21 AP-1 activity are likely different. This conclusion was based on our  
22 observation that high doses of AGN 193109 completely inhibited  
23 transactivation without substantially inhibiting anti-AP1 activity. We  
24 therefore wished to gain additional evidence to support our model for RAR\*  
25 formation mediated by AGN 193109 treatment. To accomplish this, we  
26 investigated whether AGN 193109 could potentiate the activity of the RAR  
27 specific agonist AGN 191183 in an *in vitro* transactivation assay.

28 Example 16 describes the methods used to demonstrate that AGN  
29 193109 potentiated the activity of the RAR specific agonist, AGN 191183.

1 The results of this procedure indicated that, under particular circumstances,  
2 AGN 193109 enhanced the potency of the RAR specific retinoid, and  
3 provided strong evidence that AGN 193109 promoted RAR\* formation.

#### 4 Example 16

#### 5 Potentialiation of Retinoid Effectiveness by

#### 6 AGN 193109 Coadministration

7 Hela cells were transfected using the cationic liposome-mediated  
8 transfection procedure described by Felgner et al. in *Proc. Natl. Acad. Sci.*  
9 *USA* 84:7413 (1987).  $5 \times 10^4$  cells were plated in 12 well multiwell plates and  
10 grown in DMEM supplemented with 10% FBS. Cells were cotransfected in  
11 serum free medium using LIPOFECTAMINE reagent (2 ug/well, Life  
12 Technologies, Inc.) with  $0.7 \mu\text{g}$  of the reporter plasmid MTV-TREp-Luc,  
13 containing two copies of the TREpal response element  
14 5'-TCAGGTCATGACCTGA-3' (SEQ ID NO:5) inserted into the reporter  
15 plasmid  $\Delta$ MTV-Luc (Heyman et al. in *Cell* 68:397 (1992)), and  $0.1 \mu\text{g}$  of the  
16 RAR- $\gamma$  expression plasmid pRShRAR- $\gamma$  (Ishikawa et al. *Mol. Endocrinol.*  
17 4:837 (1990)). After six-hours of transfection, cells were fed with growth  
18 medium containing charcoal extracted FBS at a final concentration of 10%.  
19 Eighteen hours after transfection, cells were treated with vehicle alone  
20 (ethanol) or AGN 193109 in ethanol at a final concentration of from  $10^{-11}$  to  
21  $10^{-8}$  M. Six hours later, AGN 191183 was added in ethanol to a final  
22 concentration of either 0,  $10^{-10}$  or  $10^{-9}$  M. Cells were harvested after eighteen  
23 hours of AGN 191183 treatment and luciferase activity was measured as  
24 described above.

25 Preliminary experiments indicated that  $10^{-9}$  M AGN 193109 was  
26 relatively ineffective at inhibiting the response to of  $10^{-9}$  M AGN 191183 in  
27 HeLa cells: This contrasted with the ability of  $10^{-9}$  M AGN 193109 to inhibit  
28  $10^{-8}$  M ATRA in CV-1 cells (Figure 2).

29 The results presented in Figure 14 supported the prediction that AGN

1 193109 stimulated the formation of RAR\*. Consistent with our  
2 characterization of the antagonist and negative hormone activities of AGN  
3 193109, treatment with AGN 193109 resulted in a biphasic dose response  
4 curve. The lowest doses of AGN 193109 ( $10^{-11}$  and  $10^{-10}$  M) resulted in a  
5 stimulation of luciferase activity over that of AGN 191183 alone. This effect  
6 suggests that RAR\*s are generated by AGN 193109. Curiously, this was also  
7 seen for AGN 193109 treatment alone, suggesting that RAR\*s can respond  
8 to an endogenous ligand. AGN 191183 is a synthetic retinoid agonist and,  
9 like ATRA, activates transcription through the RARs. Substitution of AGN  
10 191183 for ATRA in Example 7 would give qualitatively similar results (i.e.,  
11 AGN 193109 would antagonize the effect of 10 nM AGN 191183). Example  
12 16 illustrates that, while AGN 193109 can function as an antagonist of RAR  
13 agonists, dosing conditions could easily be identified wherein AGN 193109  
14 coadministration potentiated activation mediated by an RAR agonist. It is  
15 important to note that the doses of the compounds used in Example 16 are  
16 substantially lower than the doses employed in the procedure described under  
17 Example 7. We proposed that AGN 193109 treatment could lead to RAR  
18 heterogeneity RARs versus RAR\*s. The apparent heterogeneity (i.e., ability  
19 to potentiate) appears to have different windows in transactivation versus  
20 AP-1 repression. The reason that the curves are biphasic is because, with  
21 increasing amounts of AGN 193109, there is proportionately less RAR  
22 available to bind the agonist. This doesn't appear to be the case for AP-1  
23 repression and we are left to speculate that this difference must reflect two  
24 distinct mechanisms for transactivation and AP-1 repression by the same  
25 receptor species.

26 Clinical results have confirmed that some retinoids are useful for  
27 inhibiting the growth of premalignant and malignant cervical lesions.  
28 Exemplary studies supporting this conclusion have been published by Graham  
29 et al. in *West. J. Med.* 145: 192 (1986), by Lippman et al. in *J. Natl. Cancer*

1 *Inst.* 84:241 (1992), and by Weiner et al. in *Invest. New Drugs* 4:241 (1986)).

2 Similar conclusions are supported by the results of *in vitro* studies that  
3 used cultured cells to quantitate the antiproliferative effects of various  
4 retinoids. More specifically, Agarwal et al. in *Cancer Res.* 51:3982 (1991)  
5 employed the ECE16-1 cell line to model the early stages of cervical  
6 dysplasia and demonstrated that retinoic acid could inhibit epidermal growth  
7 factor (EGF) dependent cellular proliferation.

8 Example 17 describes the methods used to demonstrate that AGN  
9 193109 can antagonize the activity of the AGN 191183 retinoid agonist which  
10 inhibited proliferation of the ECE16-1 cell line.

#### 11 **Example 17**

#### 12 AGN 193109 Antagonizes the Antiproliferative Effect 13 of Retinoids in ECE16-1 Cells

14 ECE16-1 cells were seeded at a density of  $1 \times 10^4$  cells per  $\text{cm}^2$  in  
15 complete medium containing DMEM:F12 (3:1), nonessential amino acids, 5%  
16 FBS, 5  $\mu\text{g/ml}$  transferrin, 2 nM of 3,3',5 triiodothyronine (thyroid hormone or  
17 " $\text{T}_3$ "), 0.1 nM cholera toxin, 2 mM L-glutamine,  $1.8 \times 10^{-4}$  M adenine and 10  
18 ng/ml EGF. Cells were allowed to attach to plates overnight and then shifted  
19 to defined medium containing DMEM:F12 (3:1), 2 mM L-glutamine,  
20 nonessential amino acids, 0.1% bovine serum albumin,  $1.8 \times 10^{-4}$  M adenine,  
21 5  $\mu\text{g/ml}$  transferrin, 2 nM  $\text{T}_3$ , 50  $\mu\text{g/ml}$  ascorbic acid, 100  $\mu\text{g/ml}$  streptomycin,  
22 100 units/ml penicillin and 50  $\mu\text{g/ml}$  gentamicin. Defined medium (DM) was  
23 supplemented with 10 ng/ml EGF. EGF treated cells received 10 nM of the  
24 AGN 191183 retinoid agonist in combination with either 0, 0.1, 1.0, 10, 100  
25 or 1000 nM AGN 193109 or 1000 nM AGN 193109 alone. After three days  
26 of treatment, cells were harvested as described by Hembree et al. in *Cancer*  
27 *Res.* 54:3160 (1994) and cell numbers determined using a COULTER  
28 counter.

29 The results presented in Figure 15 demonstrated that ECE16-1 cells



1 proliferated in response to EGF but not in defined medium alone. This  
2 confirmed the findings published by Andreatta-van Leyen et al. in *J. Cell.*  
3 *Physio.* 160:265 (1994), and by Hembree et al. in *Cancer Res.* 54:3160 (1994).  
4 Addition of 10 nM AGN 191183 and 0 nM AGN 193109 completely inhibited  
5 EGF mediated proliferation. Thus, AGN 191183 was a potent  
6 antiproliferative retinoid. Increasing the AGN 193109 concentration from 0  
7 nM to 10 nM antagonized the AGN 191183 mediated growth inhibition by  
8 approximately 50%. A ten-fold molar excess of AGN 193109 completely  
9 reversed the antiproliferative effect of AGN 191183. Treatment of cells with  
10 1000 nM AGN 193109 alone had no effect on the EGF mediated  
11 proliferation increase. These results proved that AGN 193109 antagonized  
12 the antiproliferative effect of a retinoid but had substantially no  
13 antiproliferative activity of its own when used to treat cells representing  
14 cervical epithelium that is sensitive to growth inhibition by retinoids such as  
15 AGN 191183. Notably, there was no evidence that AGN 193109 potentiated  
16 the antiproliferative activity of the AGN 191183 agonist using the ECE16-1  
17 model system.

18 In contrast to the model system represented by the ECE16-1 cell line,  
19 there are other examples where cellular proliferation associated with cervical  
20 dysplasia cannot be inhibited by retinoid agonists. For example, Agarwal et  
21 al. in *Cancer Res.* 54:2108 (1994) described the use of CaSki cells as a model  
22 for cervical tumors that are unresponsive to retinoid therapy. As disclosed  
23 below, rather than inhibiting cell proliferation, retinoid treatment had  
24 substantially no effect on the growth rate of CaSki cells. The following  
25 Example addressed the effect of the AGN 193109 negative hormone on the  
26 proliferation rates of this cell line. The results unexpectedly proved that  
27 AGN 193109 can inhibit the proliferation of cervical tumor cells that are  
28 unresponsive to the antiproliferative effects of retinoid agonists.

29 Example 18 describes the methods used to demonstrate that AGN

1 193109 inhibited the growth of a cervical tumor cell line that did not respond  
2 to the antiproliferative effects of other retinoids such as AGN 191183.  
3 Significantly, AGN 193109 displayed antiproliferative activity in the absence  
4 of added retinoid

#### 5 Example 18

#### 6 AGN 193109 Inhibits the Proliferation Rate of CaSki

#### 7 Cervical Carcinoma-Derived Cell Line

8 We tested the effect of EGF on CaSki cell proliferation, either alone  
9 or in combination with the AGN 191183 retinoid agonist and/or the AGN  
10 193109 negative hormone at a concentration of  $10^{-6}$  M. Cell proliferation  
11 assays were performed as described above for studies involving ECE16-1  
12 cells. EGF was added to the retinoid treated cultures to give a final  
13 concentration of 20 ng/ml. Cells were treated with AGN 191183 ( $10^{-10}$  to  $10^{-6}$   
14 M) in the presence or absence of  $10^{-6}$  M AGN 193109 for a total of three  
15 days. The media was replaced with fresh media and each of the two retinoid  
16 compounds, as appropriate, every day. Cell numbers were determined using  
17 a COULTER counter as described above.

18 The results presented in Figure 16 indicated that CaSki cells were  
19 substantially refractory to the effects of a retinoid agonist and that AGN  
20 193109 exhibited antiproliferative activity in the absence of added retinoid.  
21 The presence of EGF in the culture media stimulated CaSki cell growth.  
22 This conclusion was based on comparison of the stripped bar representing no  
23 AGN 191183 and the open bar representing defined growth media ("DM")  
24 alone. AGN 191183 treatment had no antiproliferative activity on the CaSki  
25 tumor cell line. We discounted any slight increase in the cellular  
26 proliferation rate associated with the retinoid agonist, because a ten thousand  
27 fold increase in the retinoid agonist concentration was associated with only  
28 roughly a 20% increase in the proliferation rate. Thus, the AGN 191183  
29 agonist had substantially no effect on the proliferation rate of CaSki cells.

1       The results presented in Figure 16 also indicated that AGN 193109  
2 inhibited proliferation of the CaSki cervical epithelial cell line. This  
3 conclusion was based on comparison of the measurements appearing as the  
4 "0" AGN 191183 black bar and the "0" AGN 191183 stripped bar. Thus,  
5 AGN 193109 was capable of stimulating a biological response in the absence  
6 of added retinoid agonist when used to treat cervical tumor cells that were  
7 not growth inhibited by retinoid agonists such as AGN 191183.

8       Our discovery that the AGN 193109 negative hormone could inhibit  
9 cellular proliferation was consistent with a model in which unliganded RAR  
10 mediated the expression of genes that were required for proliferation. While  
11 an RAR agonist such as AGN 191183 had substantially no effect, or perhaps  
12 promoted cellular proliferation slightly, AGN 193109 had an antiproliferative  
13 effect. The AGN 193109 negative hormone likely bound RARs thereby  
14 promoting NCP association and causing the RARs to adopt an inactive  
15 conformation. According to our model, this repressed gene activity that was  
16 positively regulated by unliganded RARs. This ability of AGN 193109 to  
17 down-regulate the activity of unliganded RARs likely resulted from its ability  
18 to promote the association of RARs and NCPs.

19       Those having ordinary skill in the art will appreciate that some retinoid  
20 agonists are useful for controlling the undesirable consequences of cell  
21 growth that follows retinal detachment. After retinal detachment the retinal  
22 pigment epithelium (RPE) dedifferentiates, proliferates and migrates into the  
23 subretinal space. This process can negatively impact the success of surgical  
24 procedures aimed at retinal reattachment. Campochiaro et al. in *Invest.*  
25 *Ophthalmol & Vis. Sci.* 32:65 (1991) have demonstrated that RAR agonists such as  
26 ATRA exhibited an antiproliferative effect on the growth of primary human  
27 RPE cultures. Retinoid agonists have also been shown to decrease the  
28 incidence of retinal detachment following retinal reattachment surgery  
29 (Fekrat et al. *Ophthalmology* 102:412 (1994)). As disclosed in the following

1 Example, we analyzed the ability of the AGN 193109 negative hormone to  
2 suppress growth in primary human RPE cultures.

3 Example 19 describes the methods used to demonstrate that AGN  
4 193109 potentiated the antiproliferative effect of a retinoid antagonist in a  
5 primary culture of human retinal pigment epithelium.

### 6 Example 19

#### 7 AGN 193109 Potentiates the Antiproliferative Activity of ATRA

8 Primary cultures of human retinal pigment epithelium (RPE) were  
9 established according to the method described by Campochiaro et al. in  
10 *Invest. Ophthal & Vis. Sci.* 32:65 (1991).  $5 \times 10^4$  cells were plated in 16-mm  
11 wells of 24-well multiwell plates in DMEM (Gibco) containing 5% FBS.  
12 Cells were mock treated with ethanol vehicle alone, ATRA ( $10^{-10}$  to  $10^{-6}$  M)  
13 in ethanol, AGN 193109 ( $10^{-10}$  to  $10^{-6}$  M) in ethanol, or ATRA ( $10^{-10}$  to  $10^{-6}$   
14 M) and  $10^{-6}$  M AGN 193109. Cells were fed with fresh media containing the  
15 appropriate concentrations of these compounds every two days for a total of  
16 five days of treatment. Cells were removed from the plates by gentle  
17 digestion with trypsin and the number of cells was counted with an electronic  
18 cell counter.

19 The results presented in Figure 17 indicated that AGN 193109  
20 dramatically potentiated the antiproliferative activity of ATRA on RPE cells.  
21 Treatment of primary RPE cells with ATRA led to a dose dependent  
22 decrease in RPE cell proliferation with an approximately 40% decrease at  $10^{-6}$   
23 M ATRA relative to control cultures. AGN 193109 treatment did not  
24 substantially alter the growth rate of the RPE cells at any concentration  
25 tested in the procedure. Unexpectedly, the combination of ATRA ( $10^{-11}$  to  
26  $10^{-6}$  M) and  $10^{-6}$  M AGN 193109 had a stronger antiproliferative activity than  
27 ATRA alone. Thus, AGN 193109 cotreatment potentiated the  
28 antiproliferative effect of ATRA. More specifically, the results shown in the  
29 Figure indicated that the antiproliferative effect of  $10^{-8}$  M ATRA was

1 obtainable using only  $10^{-10}$  M ATRA in combination with  $10^{-7}$  M AGN  
2 193109. Thus, the AGN 193109 negative hormone advantageously enhanced  
3 the antiproliferative activity of ATRA by approximately 100 fold.

4 In an independent experiment, comparison of the antiproliferative  
5 effect of ATRA ( $10^{-11}$  to  $10^{-6}$  M) with that of ATRA and  $10^{-6}$  M AGN 193109  
6 again demonstrated the apparent increase in sensitivity of primary RPE cells  
7 to ATRA in the presence of AGN 193109. In this system, AGN 193109  
8 neither functioned as a retinoid antagonist nor exhibited an antiproliferative  
9 effect when used alone. However, AGN 193109 coadministration potentiated  
10 the antiproliferative activity of the retinoid agonist.

11 AGN 193109 was tested for its ability to potentiate the anti-  
12 proliferative effect of 13-*cis* retinoic acid (13-*cis* RA) in primary RPE cultures  
13 using conditions and techniques to measure RPE cell proliferation described  
14 above. Notably, 13-*cis* RA is clinically significant. More particularly, 13-*cis*  
15 RA is useful in the treatment of several disease states, including acne (Peck  
16 et al. *N. Engl. J. Med.* 300:329 (1977); Jones et al. *Br. J. Dermatol.* 108:333  
17 (1980)), and squamous cell carcinoma of the skin and cervix in combination  
18 treatment with interferon  $2\alpha$  (Lippman et al. *J. Natl. Cancer Inst.* 84:241  
19 (1992); Moore et al. *Seminars in Hematology* 31:31 (1994)).

20 The results presented in Figure 18 indicated that both 13-*cis* RA ( $10^{-12}$   
21 to  $10^{-6}$ M) and ATRA ( $10^{-12}$  to  $10^{-6}$ M) effectively inhibited RPE cell growth.  
22 Notably, the 13-*cis* isomer was approximately two orders of magnitude less  
23 effective in this assay when compared with ATRA. Similar to the results  
24 obtained using coadministration of AGN 193109 and ATRA (above),  
25 coadministration of AGN 193109 (either  $10^{-8}$  or  $10^{-6}$ M) with 13-*cis* RA ( $10^{-12}$   
26 to  $10^{-6}$ M) dramatically increased the potency of 13-*cis* RA in mediating  
27 repression of RPE cell proliferation. In contrast to treatment with 13-*cis* RA  
28 alone, coadministration of AGN 193109 enhanced the potency of 13-*cis* RA.  
29 Thus, AGN 193109 potentiated the antiproliferative activity of 13-*cis* RA.

1       We next tested the ability of AGN 193109 to potentiate the activities  
2 of other nuclear receptor hormones in primary RPE cell cultures.  
3 Dexamethasone, a synthetic glucocorticoid receptor agonist, is one member  
4 of a class of compounds that have been used clinically for their potent anti-  
5 inflammatory and immunosuppressive properties. Thyroid hormone (T3;  
6 3,3',5'-Triiodothyronine) is a natural thyroid hormone receptor agonist used  
7 primarily for hormone replacement therapy in the treatment of  
8 hypothyroidism. Methods used in these experiments were identical to those  
9 described above for procedures employing ATRA and 13-*cis* RA.

10       The results of these procedures indicated that coadministration of  
11 AGN 193109 and the nuclear receptor agonists potentiated the  
12 antiproliferative activities of the nuclear receptor agonists. More specifically,  
13 the results presented in Figure 19 showed that single-agent treatment of RPE  
14 cells with either dexamethasone ( $10^{-11}$  to  $10^{-6}$ M) or ATRA ( $10^{-12}$  to  $10^{-6}$ M)  
15 was substantially unable to inhibit RPE cell proliferation. However,  
16 treatment of RPE cells with dexamethasone ( $10^{-11}$  to  $10^{-6}$ M) and either  $10^{-8}$  or  
17  $10^{-6}$ M AGN 193109 repressed RPE cell proliferation to an extent that  
18 approximated the inhibition caused by treatment with ATRA. Similarly, the  
19 results presented in Figure 20 indicated that AGN 193109 potentiated the  
20 antiproliferative activity of thyroid hormone. Similar to the results obtained  
21 using dexamethasone, the proliferation of RPE cells was refractory to single-  
22 agent treatment with thyroid hormone ( $10^{-11}$  to  $10^{-6}$ M). However, co-  
23 treatment of RPE cells with thyroid hormone ( $10^{-11}$  to  $10^{-6}$ M) and AGN  
24 193109 (either  $10^{-8}$  or  $10^{-6}$ M) inhibited RPE cell proliferation in a thyroid  
25 hormone dependent manner. We concluded that AGN 193109 rendered  
26 primary RPE cultures sensitive to the anti-proliferative effects of these  
27 nuclear-receptor agonists. The mechanism by which AGN 193109 mediated  
28 these effects likely involved modulation of NCP/RAR interactions.

29       We additionally examined the effect of AGN 193109 on the expression

1 of marker genes in other experimental systems that were sensitive to retinoid  
2 agonists. Both the MRP8 and stromelysin genes are known to be inhibited  
3 by retinoid agonists in a variety of biological systems. For example,  
4 Wilkinson et al. in *J. Cell Sci.* 91:221 (1988) and Madsen et al. in *J. Invest.*  
5 *Dermatol.* 99:299 (1992) have disclosed that MRP8 gene expression was  
6 elevated in psoriasis. Conversely, MRP8 gene expression was repressed by  
7 the retinoid agonist AGN 190168 in human psoriatic skin (Nagpal et al.,  
8 submitted 1995), in human keratinocyte raft cultures (Chandraratna et al. *J.*  
9 *Invest. Dermatol.* 102:625 (1994)) and in cultured human newborn foreskin  
10 keratinocytes (Thacher et al. *J. Invest. Dermatol.* 104:594 (1995)). Nagpal et  
11 al. in *J. Biol. Chem.* 270:923 (1995) have disclosed that stromelysin mRNA  
12 levels were repressed by retinoid agonists such as AGN 190168 in cultured  
13 human newborn foreskin keratinocytes. We analyzed the regulated  
14 expression of these genes following treatment of cultured human newborn  
15 foreskin keratinocytes with either the AGN 191183 retinoid agonist or AGN  
16 193109.

17 Example 20 describes the methods used to demonstrate that AGN  
18 193109 inhibited MRP-8 expression in cultured keratinocytes.

#### 19 Example 20

#### 20 AGN 193109 Inhibits MRP-8 Expression 21 in Keratinocytes

22 Primary foreskin keratinocytes were isolated according to the  
23 procedure described by Nagpal et al. in *J. Biol. Chem.* 270:923 (1995) and  
24 cultured in keratinocyte growth medium (KGM) that was purchased from  
25 Clonetics. After 3 days of treatment with AGN 191183 ( $10^{-7}$  M) or AGN  
26 193109 ( $10^{-6}$  M), total cellular RNA was isolated from treated and control  
27 keratinocytes according to standard methods. The mRNA was reverse  
28 transcribed into cDNA which then served as the template in a PCR  
29 amplification protocol using primers specific for either the glyceraldehyde

1 phosphate dehydrogenase (GAPDH) housekeeping gene or MRP-8. The  
2 GAPDH primers had the sequences 5'-  
3 CCACCCATGGCAAATTCCATGGCA-3' (SEQ ID NO:6) and 5'-  
4 TCTAGACGGCAGGTCAGGTCCACC-3' (SEQ ID NO:7). The MRP-8  
5 primers had the sequences 5'-ACGCGTCCGGAAGACCTGGT-3' (SEQ ID  
6 NO:8) and 5'-ATTCTGCAGGTACATGTCCA-3' (SEQ ID NO:9). An  
7 aliquot from the MRP-8 amplification reaction (10  $\mu$ l) was removed after  
8 every cycle of PCR amplification starting from 12 cycles and ending at 21  
9 cycles. Similarly, an aliquot of the GAPDH amplification reaction was  
10 removed after every PCR cycle starting at 15 cycles and ending at 24 cycles.  
11 The samples were electrophoresed on 2% agarose gels and the separated  
12 amplification products detected by ethidium bromide staining. The staining  
13 intensity of the amplification products served as a quantitative measure of the  
14 amount of starting mRNA specific for the given primer set.

15 The results of this procedure indicated that both AGN 191183 and  
16 AGN 193109 independently inhibited MRP-8 expression in keratinocytes.  
17 The intensity of the stained GAPDH amplification product was substantially  
18 equivalent in the lanes of the gel representing starting material isolated from  
19 control, AGN 191183, and AGN 193109 treated keratinocytes. Weak bands  
20 representing the GAPDH amplification product were first detectable in lanes  
21 corresponding to samples removed after 18 cycles of PCR amplification. The  
22 equivalent staining intensities among the various lanes of the gel indicated  
23 that equivalent masses of starting material were used for all samples.  
24 Accordingly, differences in the intensities of stained bands representing  
25 MRP-8 amplification products were indicative of differences in MRP-8  
26 mRNA expression among the various starting samples. As expected, the  
27 MRP-8 amplified signal was inhibited in AGN 191183 ( $10^{-7}$  M) treated  
28 cultures relative to an untreated control. AGN 193109 ( $10^{-6}$  M) treatment of  
29 cultured keratinocytes also repressed MRP8 expression as judged by lower



1 intensity of stained amplification product.

2 As illustrated in the following Example, AGN 193109 also inhibited  
3 expression of a second marker gene in keratinocytes. Nagpal et al. in *J. Biol.*  
4 *Chem.* 270:923 (1995) disclosed that stromelysin mRNA expression was  
5 down-regulated by RAR specific agonists in cultured newborn human  
6 foreskin keratinocytes. Nicholson et al. (*EMBO J.* 9:4443 (1990)) disclosed  
7 that an AP-1 promoter element played a role in the retinoid-dependent  
8 negative regulation of the stromelysin-1 gene. Thus, it was of interest to  
9 determine whether AGN 193109 could alter the expression of this gene.

10 Example 21 describes the methods used to demonstrate that AGN  
11 193109 inhibited stromelysin-1 gene expression in the absence of an  
12 exogenously added retinoid agonist.

#### 13 Example 21

##### 14 AGN 193109 Inhibits Stromelysin-1 Expression 15 in Cultured Keratinocytes

16 Primary foreskin keratinocytes were either mock treated or treated for  
17 24 hours with the RAR agonist AGN-191183 ( $10^{-7}$  M), or AGN 193109 ( $10^{-6}$   
18 M). Total RNA prepared from mock-treated and retinoid-treated  
19 keratinocytes was reverse transcribed and the resulting cDNA was PCR  
20 amplified using  $\beta$ -actin or stromelysin-1 oligo primers exactly as described by  
21 Nagpal et al. in *J. Biol. Chem.* 270:923 (1995)), the disclosure of which has  
22 been incorporated by reference. A sample (10  $\mu$ l) from the PCR  
23 amplification reaction was removed after every three cycles starting from 18  
24 cycles of PCR amplification. The sample was electrophoresed on a 2%  
25 agarose gel and detected after ethidium bromide staining.

26 Results of these procedures indicated that AGN 193109 inhibited  
27 stromelysin-1 gene expression in the absence of an exogenously added  
28 retinoid agonist. More specifically, ethidium-stained bands representing  $\beta$ -  
29 actin amplification products were easily detectable the agarose gels after 18

1 cycles of PCR. While all band intensities increased with additional cycles of  
2 the amplification reaction, stained bands were somewhat less intense in  
3 samples representing AGN 191183 treated cells. This indicated that a slightly  
4 lesser amount of RNA must have been present in the starting samples  
5 corresponding to cells treated with AGN 191183. The results also indicated  
6 that stromelysin-1 mRNA was detectable in mock-treated keratinocytes  
7 starting at 33 cycles of PCR amplification. As expected, stromelysin-1  
8 mRNA expression was inhibited after AGN 191183 ( $10^{-7}$  M) treatment as  
9 judged by the weaker band intensity on when compared with samples derived  
10 from mock-treated samples. When normalized to the intensities of the  $\beta$ -  
11 actin amplification products, and consistent with the results obtained in  
12 measurements of MRP-8 expression, AGN 193109 ( $10^{-6}$  M) treatment of  
13 keratinocytes resulted in down-regulation of stromelysin-1 mRNA levels.  
14 Indeed, the down-regulation stimulated by AGN 193109 treatment was  
15 indistinguishable from the down-regulation caused by treatment of  
16 keratinocytes with the RAR agonist AGN 191183.

17 As disclosed herein, AGN 193109 can have any of three possible  
18 effects with respect to modulating the activity of a coadministered steroid  
19 superfamily agonist. First, AGN 193109 may have no effect. Second, AGN  
20 193109 may antagonize the effect of the agonist, thereby leading to a  
21 decrease in the activity of the agonist. Finally, AGN 193109 may potentiate  
22 the activity of the agonist, thereby leading to a stimulation of the measured  
23 effect produced by the agonist.

24 Compounds having activities that can be modulated by AGN 193109  
25 include retinoid receptor agonists and agonists which bind to other members  
26 of the steroid receptor superfamily. This latter category of agonists includes  
27 vitamin D receptor agonists, glucocorticoid receptor agonists and thyroid  
28 hormone receptor agonists. Peroxisome proliferator-activated receptors,  
29 estrogen receptor and orphan receptors having presently unknown ligands

1 may also be potentiated by AGN 193109. In the case where the steroid  
2 superfamily agonist is an RAR agonist, AGN 193109 may either antagonize  
3 or potentiate the activity of that agonist. In the case where the agonist used  
4 in combination with AGN 193109 is a compound that can bind to a nuclear  
5 receptor other than an RAR, coadministration of AGN 193109 will either  
6 have no effect or will sensitize of the system to the agonist so that the activity  
7 of the agonist is potentiated.

8 A generalized exemplary procedure for determining which of the three  
9 possible activities AGN 193109 will have in a particular system follows. This  
10 description illustrates each of the possible outcomes for AGN 193109  
11 coadministration with a steroid receptor superfamily agonist. Biological  
12 systems useful for assessing the ability of AGN 193109 to modulate the  
13 activity of a nuclear receptor agonist include but are not limited to:  
14 established tissue culture cell lines, virally transformed cell lines, *ex-vivo*  
15 primary culture cells and *in vivo* studies utilizing living organisms.  
16 Measurement of the biological effect of AGN 193109 in such systems could  
17 include determination of any of a variety of biological endpoints. These  
18 endpoints include: analysis of cellular proliferation, analysis of programmed  
19 cell death (apoptosis), analysis of the differentiation state of cells via gene  
20 expression assays, analysis of the ability of cells to form tumors in nude mice  
21 and analysis of gene expression after transient or stable introduction of  
22 reporter gene constructs.

23 For illustrative purposes, an mRNA species designated as mRNA "X"  
24 is expressed from gene "X" in primary cultured "Y" cells isolated from the  
25 organ "Z." Under standard culture conditions, where several "Y" cell genetic  
26 markers are maintained, including expression of gene "X", addition of a  
27 retinoid agonist leads to a decrease in the abundance of "X" mRNA.  
28 Analysis of gene X expression can be assessed via isolation of cellular mRNA  
29 and measurement of the abundance of X mRNA levels via polymerase chain

1 reaction, ribonuclease protection or RNA blotting procedures such as  
2 Northern analyses. After isolation from organ Z, primary Y cells are  
3 cultured in an appropriate growth medium. The primary cultures are then  
4 plated into tissue culture plates for expansion of the cell population. This  
5 step facilitates separation of the cells into four sample groups so that various  
6 doses of the retinoid agonist and AGN 193109 can be delivered. The first  
7 group will be a control, receiving vehicle only. The second group will receive  
8 the RAR agonist, retinoic acid, delivered in ethanol, in amounts sufficient to  
9 provide final concentrations in the range of from  $10^{-11}$  to  $10^{-6}$  M. The lowest  
10 dose may need to be empirically determined depending on the sensitivity of  
11 the system. Such determinations fall within the scope of routine  
12 experimentation for one having ordinary skill in the art. The third group will  
13 receive both the nuclear receptor agonist at the same doses used for treating  
14 the cells of group 2, and a constant dose of AGN 193109. The dose of AGN  
15 193109 used for treating the cells of group 3 will also need to be determined  
16 empirically, but should approximate the affinity constant ( $K_d$ ) of AGN  
17 193109 for the RAR subtypes (i.e., at least  $10^{-8}$  M). The fourth group will  
18 receive AGN 193109 at doses minimally including that used for agonist  
19 coadministration in group 3. An alternative to this dosing regimen would  
20 substitute AGN 193109 for the retinoid agonist described in the foregoing  
21 example, as specified in group 2, and a constant dose of retinoid agonist in  
22 place of AGN 193109, as specified in groups 3 and 4. After a suitable  
23 incubation period, cells should be harvested in a manner suitable for  
24 determination of the biological endpoint being measured as an indicator of  
25 agonist activity.

26 For example, analysis of the effect of AGN 193109 on retinoic acid  
27 dependent regulation of gene expression would involve comparison of the  
28 abundance of the mRNA species X in the mRNA pool harvested from cells  
29 treated according to each of the four protocols described above. RNA

1 derived from control cells will serve to determine the baseline expression of  
2 X mRNA and will represent a condition corresponding to no repression.  
3 Comparison of this level with that measured in the mRNA pool derived from  
4 cells treated with retinoic acid will allow for determination of the effect of  
5 this agonist on gene expression. Quantitated levels of the repression of  
6 specific mRNAs resulting from retinoic acid treatment can then be compared  
7 with mRNA abundances from cells treated in parallel with either AGN  
8 193109 alone or AGN 193109 in combination with retinoic acid. While this  
9 generalized example illustrates an analysis of the effect of coadministered  
10 AGN 193109 on the expression of a gene repressed by a retinoid agonist, the  
11 example could alternatively have described analysis of the effect of  
12 coadministered AGN 193109 on a gene that was induced by a retinoid  
13 agonist. The critical feature for determining whether AGN 193109 will  
14 behave as an agonist, as a negative hormone or have no effect in a particular  
15 system will involve quantitative comparison of the magnitude of the effect in  
16 the presence and absence of AGN 193109.

17 An example in which AGN 193109 potentiated the activity of a  
18 coadministered agonist would be a case in which AGN 193109 cotreatment  
19 with retinoic acid resulted in a level of X mRNA expression that is further  
20 repressed relative to the level measured in cells treated with retinoic acid  
21 alone. More specifically, comparison of the dose response curve of the  
22 biological effect (i.e., repression of X mRNA abundance) plotted on the Y-  
23 axis versus the dose of the agonist (logarithmic scale) on the X-axis would  
24 allow comparison of agonist-mediated repression of X mRNA abundance in  
25 the presence and absence of AGN 193109 cotreatment. The ability of AGN  
26 193109 to sensitize the biological response to the agonist, thereby potentiating  
27 the activity of the agonist, will be indicated by a leftward shift in the dose  
28 response curve. More specifically, in the presence of AGN 193109 less  
29 agonist would be required to obtain the same biological effect obtainable

1 using the agonist alone.

2 An example of AGN 193109 mediating antagonism of a coadministered  
3 agonist would be a case in which AGN 193109 cotreatment with retinoic acid  
4 resulted in a level of X mRNA expression that is less repressed compared to  
5 that measured in cells treated with retinoic acid alone. Comparison of dose  
6 response curves of X mRNA repression versus log dose of agonist in the  
7 presence and absence of AGN 193109 will demonstrate a shift to the right in  
8 the dose response curve. More specifically, in the presence of AGN 193109,  
9 more agonist will be necessary to obtain the same biological effect obtainable  
10 with single agent treatment with the agonist alone.

11 The above examples wherein AGN 193109 mediates either antagonism  
12 or potentiation describe experimental outcomes for coadministration of AGN  
13 193109 with a retinoid agonist. If, however, the agonist coadministered with  
14 AGN 193109 is an agonist capable of binding and activating a member of the  
15 steroid receptor superfamily other than an RAR, then instead of antagonizing  
16 the agonist, it becomes possible that AGN 193109 would have no effect on  
17 the activity of the agonist. If AGN 193109 cotreatment with such an agonist  
18 results in a level of mRNA expression which is equal to that measured in  
19 cells treated with agonist alone, then AGN 193109's ability to affect the  
20 availability of NCPs via promotion of RAR:NCP associations will be silent in  
21 this system. This would be an example wherein AGN 193109 has no effect  
22 on a coadministered agonist.

### 23 Example of Antagonism

24 The method disclosed in the above generalized example for  
25 determining the effect of AGN 193109 coadministered with a retinoid agonist  
26 is exemplified by the procedure described under Example 7. CV-1 cells  
27 cotransfected with one of the three retinoic acid receptors and the retinoid  
28 agonist inducible MTV-TREp-Luc reporter construct were dosed with either  
29 ethanol (control, group 1), AGN 193109 at final concentrations of from  $10^{-9}$

1 to  $10^{-6}$  M (group 2), AGN 193109 at final concentrations of from  $10^{-9}$  to  $10^{-6}$   
2 M coadministered with retinoic acid at  $10^{-8}$  M (group 3), or retinoic acid ( $10^{-8}$   
3 M, group 4). Comparison of the luciferase activity of group 1 with that of  
4 group 4 allowed determination of the level of retinoid agonist induced  
5 expression of the luciferase reporter gene in the absence of added AGN  
6 193109. Comparison of luciferase reporter gene expression in cells of group  
7 3 with that measured in cells of group 4 indicated that AGN 193109 behaved  
8 as an antagonist of the retinoid agonist in this system.

9 Example of Antagonism

10 The method disclosed in the generalized example for determining the  
11 effect of AGN 193109 coadministered with a retinoid agonist was similarly  
12 used to determine in Example 17 that AGN 193109 functioned as an  
13 antagonist of a retinoid agonist-mediated repression of EGF-stimulated  
14 cellular proliferation in ECE-16-1 transformed cervical epithelial cells. In  
15 this procedure, treatments of ECE-16-1 cells included a control sample  
16 treated with EGF alone (group 1), a sample treated with the combination of  
17 EGF and AGN 193109 at a final concentration of  $10^{-6}$  M (group 2), a sample  
18 treated with the combination of EGF and AGN 193109 at final  
19 concentrations of from  $10^{-10}$  to  $10^{-6}$  M coadministered with a single dose of  
20 the retinoid agonist AGN 191183 at  $10^{-8}$  M (group 3), and a sample treated  
21 with the combination of EGF and AGN 191183 at  $10^{-8}$  M (group 4). After  
22 three days of treatment, cellular proliferation rates were determined.  
23 Determination that the cells had been stimulated to proliferate by EGF was  
24 possible because an additional control treatment was included wherein cells  
25 were exposed to defined medium that did not contain EGF. Comparison of  
26 the number of cells in group 1 with the number of cells in group 4 allowed  
27 for determination that RAR agonist AGN 191183 repressed the EGF-  
28 stimulated proliferation of ECE-16-1 cells. Comparison of group 3 with  
29 group 4 indicated that AGN 193109 antagonized the activity of the RAR

1 agonist in this system.

2 Example of Potentiation

3       The method disclosed in the generalized example for determining the  
4 effect of AGN 193109 coadministered with a retinoid agonist was also used in  
5 Example 14 to determine that AGN 193109 potentiated the activity of a  
6 nuclear receptor agonist in HeLa cells transfected with the 1,25-  
7 dihydroxyvitamin D<sub>3</sub> inducible MTV-VDRE-Luc reporter gene. Treatments  
8 of transfected cells included vehicle alone (control, group 1), 1,25-  
9 dihydroxyvitamin D<sub>3</sub> at final concentrations of from 10<sup>-10</sup> to 10<sup>-7</sup> M (group 2),  
10 1,25-dihydroxyvitamin D<sub>3</sub> at final concentrations of from 10<sup>-10</sup> to 10<sup>-7</sup> M  
11 coadministered with AGN 193109 at a final concentration of either 10<sup>-8</sup> or 10<sup>-7</sup>  
12 M (group 3), and AGN 193109 as a single agent treatment at a final  
13 concentration of either 10<sup>-8</sup> or 10<sup>-7</sup> M (group 4). Comparison of the  
14 luciferase activity measured in group 1 (control) cells with that of group 2  
15 cells allowed for determination that 1,25-dihydroxyvitamin D<sub>3</sub> stimulated  
16 luciferase activity was dose-dependent. Comparison of luciferase activity  
17 measured in cells of group 4 (AGN 193109 single agent treatment) with that  
18 measured in cells of group 3 (AGN 193109 coadministration) similarly  
19 allowed for determination of dose-dependent 1,25-dihydroxyvitamin D<sub>3</sub>  
20 stimulated luciferase activity in the presence of a given concentration of AGN  
21 193109. In this instance, the zero value represented the luciferase activity in  
22 cells treated with AGN 193109 alone (group 4). Such a dosing regimen  
23 allowed for comparison of three 1,25-dihydroxyvitamin D<sub>3</sub> dose response  
24 curves. Comparison of the dose response curve of 1,25-dihydroxyvitamin D<sub>3</sub>  
25 in the absence of AGN 193109 with the curve representing coadministration  
26 of AGN 193109 (either 10<sup>-8</sup> or 10<sup>-7</sup> M) demonstrated potentiation of the  
27 agonist activity as evidenced by a leftward shift in the half-maximal response.

28 Example of Potentiation

29       The method disclosed in the generalized example for determining the



1 effect of AGN 193109 coadministered with a retinoid agonist was further  
2 used to determine in Example 19 that AGN 193109 potentiated the  
3 antiproliferative activity of an RAR agonist in primary cultures of human  
4 retinal pigment epithelium cells. Treatments of cells included: ethanol  
5 vehicle alone (group 1), retinoic acid at final concentrations of from  $10^{-10}$  to  
6  $10^{-6}$  M (group 2), retinoic acid at final concentrations of from  $10^{-10}$  to  $10^{-6}$  M  
7 coadministered with  $10^{-6}$  M AGN 193109 (group 3), and AGN 193109 alone  
8 at final concentrations of from  $10^{-10}$  to  $10^{-6}$  M (group 4). Comparison of  
9 assay results obtained using cells of groups 1 and 2 allowed for determination  
10 of the dose dependent inhibition of proliferation of these cells by retinoic  
11 acid. Similarly, comparison of results obtained using cells of group 3 with  
12 those of group 1 allowed for determination of the dose dependent inhibition  
13 of proliferation of these cells by retinoic acid in the presence of  
14 coadministered AGN 193109. Group 4 demonstrated the inability of AGN  
15 193109 to substantially alter the proliferation rate of these cells when used as  
16 a single treatment agent. Comparison of the dose response curves of retinoic  
17 acid-mediated repression of cellular proliferation generated in groups 2 and 3  
18 provided the basis for the conclusion that AGN 193109 sensitized primary  
19 RPE cells to the antiproliferative effects of the RAR agonist, thereby  
20 potentiating the activity of the RAR agonist.

21 As indicated above, Agarwal et al., in *Cancer Res.* 54:2108 (1994)),  
22 showed that CaSki cell growth, unlike the growth of HPV immortalized ECE-  
23 16-1 cells, was not inhibited by treatment with retinoid agonists. As disclosed  
24 herein, we unexpectedly found that CaSki cell growth was inhibited by AGN  
25 193109 in the absence of a retinoid agonist. The following Example  
26 illustrates how AGN 193109 can be used to inhibit the growth of CaSki cell  
27 tumors *in vivo*.

## 28 Example 22

### 29 Inhibition of CaSki Cell Tumor Growth in Nude Mice

### Following Administration of AGN 193109

1  
2 1 X 10<sup>6</sup> CaSki cells are injected into each of a panel of nude mice.  
3 Tumor formation is assessed using techniques that will be familiar to one  
4 having ordinary skill in the art. After injection, mice are randomly divided  
5 into control and test groups. The control group receives a placebo. The test  
6 group is administered with AGN 193109. Animals administered with the  
7 placebo receive intragastric intubation of corn oil. The test animals receive  
8 20  $\mu$ Mol/kg AGN 193109 in corn oil daily for the period of the treatment.  
9 Tumor volume is measured in cubic milliliters using graduated calipers.  
10 Tumor volume is plotted as function of time. Mice receiving AGN 193109  
11 exhibit tumors which are significantly reduced in their growth rate as  
12 compared to tumors in control mice as judged by tumor size and number  
13 over the period of the study. This result provides an *in vivo* demonstration  
14 that AGN 193109 inhibits the growth of an advanced cervical carcinoma that  
15 is resistant to therapy comprising administration of a retinoid agonist.

16 As indicated above, CaSki cells are a model of cervical tumors that are  
17 not responsive to retinoid agonist therapy. However, herein we have  
18 disclosed that CaSki cell growth was inhibited by AGN 193109 in the absence  
19 of treatment with a retinoid agonist. The ability of AGN 193109 to inhibit  
20 the proliferation of CaSki cells suggested that AGN 193109 could be used to  
21 therapeutically treat cervical carcinomas that are insensitive to retinoid  
22 agonist therapy. The following Example illustrates one method that can be  
23 used to assess the therapeutic potential of AGN 193109 in the treatment of a  
24 cervical carcinoma.

### **Example 23**

#### Assessing the Therapeutic Potential of AGN 193109 in Patients Having Cervical Carcinoma

25  
26  
27  
28 A patient presenting with an advanced cervical carcinoma is first  
29 identified. A cervical biopsy is obtained according to methods that will be

1 familiar to one having ordinary skill in the art. Cells from the explanted  
2 tumor are propagated in tissue culture according to standard techniques to  
3 provide cell numbers sufficient to allow division into three sample groups.  
4 Culture conditions described by Agarwal et al. in *Cancer Res.* 54:2108 (1994)  
5 are employed for this purpose. The first group is reserved as a control and  
6 receives vehicle alone (ethanol). The second group is treated with the RAR  
7 agonist retinoic acid at a concentration of from  $10^{-10}$  to  $10^{-6}$  M. The third  
8 group is treated with AGN 193109 at doses ranging from  $10^{-10}$  to  $10^{-6}$  M.  
9 Cells are fed with fresh growth medium daily and are provided with the  
10 retinoids described above as appropriate for each sample group. Cells are  
11 counted after three days using an electric cell counter. Comparison of the  
12 number of cells in control cultures with the number of cells in retinoic acid  
13 treated cultures indicates the RAR agonist does not substantially inhibit the  
14 growth rate of the cultured cervical carcinoma cells. In contrast, cells treated  
15 with AGN 193109 exhibit a dose-dependent decrease in cell number when  
16 compared with cell counts in the control group. This result, wherein AGN  
17 193109 treatment inhibits cultured cervical carcinoma cell proliferation,  
18 indicates that AGN 193109 will be a useful therapeutic agent for treating  
19 cervical carcinoma patients having metastatic disease.

20 Cervical carcinoma patients having undergone surgery for the removal  
21 of primary tumors and who present with metastatic disease are enlisted in a  
22 randomized clinical study seeking to demonstrate the therapeutic benefit of  
23 AGN 193109 in this indication. Patients are divided into two groups. The  
24 first group is a control group while members of the second group are treated  
25 with AGN 193109. AGN 193109 is combined with a pharmaceutically  
26 acceptable excipient to produce a composition suitable for systemic  
27 administration, all according to techniques that will be familiar to one having  
28 ordinary skill in the art. The control group is administered a placebo  
29 formulation and the experimental group is administered with the formulation

1 containing the AGN 193109 negative hormone. Dosing of patients is at the  
2 maximum tolerated dose and is performed every other day for a period of  
3 from three months to one year. The outcome of the study is quantified via  
4 measurement of disease-free survival over time. Individuals receiving AGN  
5 193109 display a significant increase in disease-free survival, including a  
6 disproportionate number of patients displaying complete remission of their  
7 metastatic disease. This result indicates that AGN 193109 has therapeutic  
8 utility for *in vivo* treatment of cervical carcinomas that are unresponsive to  
9 the antiproliferative effects of retinoid agonists, such as retinoic acid.

10 As disclosed above, AGN 193109 potentiated the antiproliferative  
11 activity of RAR agonists in primary cultures of human retinal pigment  
12 epithelium cells. Accordingly, coadministration of AGN 193109 with an  
13 RAR agonist *in vivo* is reasonably expected to increase the therapeutic index  
14 of the agonist because a lesser amount of the RAR agonist will be required  
15 to obtain the same therapeutic endpoint. Additionally, AGN 193109 has  
16 been demonstrated to sensitize primary cultures of human retinal pigment  
17 epithelium cells to the antiproliferative effects of glucocorticoid and thyroid  
18 hormone receptor agonists. The following rabbit model of PVR will be  
19 utilized in two separate studies to demonstrate the increased therapeutic  
20 index obtained via coadministration of AGN 193109 with an RAR agonist  
21 (13-cis retinoic acid) or a thyroid hormone receptor agonist, respectively.  
22 Notably, the rabbit model of retinal redetachment published by Sen et al. in  
23 *Arch. Ophthalmol.* 106:1291 (1988), has been used to demonstrate that retinoid  
24 agonists which inhibit proliferation of primary RPE cells *in vitro* also inhibit  
25 the frequency of retinal detachment *in vivo* (Araiz et al. *Invest. Ophthalmol.*  
26 34:522 (1993)). Thus, with respect to their use as therapeutics in the  
27 prevention of retinal detachment, a correlation between the *in vitro* and *in*  
28 *vivo* activities of retinoid agonists has already been established. The following  
29 Examples illustrate how AGN 193109 can be used in therapeutic applications

1 directed at preventing retinal detachment.

2 **Example 24**

3 Use of AGN 193109 to Increase the Therapeutic Potential of  
4 Steroid Superfamily Receptor Agonists in the Treatment  
5 of Proliferative Vitreoretinopathy (PVR)

6 In a first study, human RPE cells are injected into the vitreous cavity  
7 of rabbit eyes according to the method described by Sen et al. in *Arch.*  
8 *Ophthalmol.* 106:1291 (1988). After intravitreal injection, the rabbits are  
9 divided into five groups. The first group (control) will receive vehicle alone  
10 by intravitreal injection. The second group receives retinoic acid as single  
11 agent treatment (100  $\mu$ g) by intravitreal injection. The third group receives  
12 AGN 193109 as a single agent treatment (100  $\mu$ g) by intravitreal injection.  
13 The fourth group receives by intravitreal injection the RAR agonist (retinoic  
14 acid) at a dose one-tenth the amount administered to group 2 (10 $\mu$ g). The  
15 fifth group receives the combination of AGN 193109 (100  $\mu$ g) and retinoic  
16 acid (10 $\mu$ g) by intravitreal injection. Animals receive a single intravitreal  
17 injection of the appropriate treatment one day after intravitreal injection of  
18 human RPE cells. Rabbits are examined by indirect ophthalmoscopy on days  
19 7, 14 and 28, and are graded for the frequency and severity of tractional  
20 retinal detachment. Rabbits from the group injected with 100  $\mu$ g retinoic  
21 acid exhibit a significantly reduced frequency and severity of retinal  
22 detachment compared to control rabbits or rabbits receiving either AGN  
23 193109 or retinoic acid (10 $\mu$ g) alone. Rabbits in the group administered with  
24 the combination of AGN 193109 and retinoic acid (10 $\mu$ g) exhibit significantly  
25 reduced frequency and severity of retinal detachment as compared to those in  
26 groups either control, AGN 193109 or retinoic acid (10 $\mu$ g). This result  
27 demonstrates that AGN 193109 improves the therapeutic index of the RAR  
28 agonist retinoic acid in an *in vivo* model of PVR.

29 In a second study, rabbits are first provided with an injection of human

1 RPE cells into the vitreous cavity of the eye, and then divided into four  
2 groups. The first group (control) receives vehicle alone by intravitreal  
3 injection. The second group receives thyroid hormone as single agent  
4 treatment (100  $\mu$ g) by intravitreal injection. The third group is administered  
5 with AGN 193109 as a single agent treatment (100  $\mu$ g) by intravitreal  
6 injection. The fourth group is administered with the combination of AGN  
7 193109 (100  $\mu$ g) and thyroid hormone (100  $\mu$ g). Rabbits are examined by  
8 indirect ophthalmoscopy on days 7, 14 and 28, and graded for the frequency  
9 and severity of tractional retinal detachment. Comparison of the frequency  
10 and severity of retinal detachment in the four groups demonstrates that single  
11 agent treatment with either AGN 193109 or thyroid hormone does not inhibit  
12 retinal detachment when compared with control rabbits. In contrast, the  
13 group of rabbits administered with the combination of AGN 193109 and  
14 thyroid hormone exhibit significantly reduced incidence and severity of retinal  
15 detachment. This result demonstrates that AGN 193109 improves the  
16 therapeutic index of thyroid hormone in an *in vivo* model of PVR.

17 ——— The following Example illustrates how AGN 193109 can be used to  
18 enhance the therapeutic index of an RAR agonist used to treat human  
19 patients following retinal reattachment surgery.

#### 20 Example 25

#### 21 Increasing the Therapeutic Index of RAR Agonist

#### 22 13-cis Retinoic Acid

23 A population of adult volunteers having retinal detachment resulting  
24 from PVR is first identified. Individuals undergo surgical repair of the  
25 detachments using techniques that are standard in the art. The patients are  
26 then divided into five groups. The control group consists of patients who  
27 undergo surgical repair of the retinal detachment and do not receive any  
28 retinoid compound. The second group receives 40 mg oral 13-cis retinoic  
29 acid twice daily for four weeks postoperatively. The third group receives 40

1 mg oral AGN 103109 twice daily for four weeks postoperatively. The fourth  
2 group receives 4 mg oral 13-cis retinoic acid twice daily for four weeks  
3 postoperatively. The fifth group receives 40 mg oral AGN 193109 in  
4 combination with 4 mg oral 13-cis retinoic acid twice daily for four weeks  
5 postoperatively. The treatment protocol and assessment of drug efficacy is  
6 performed essentially as described by Fekrat et al. in *Ophthalmology* 102:412  
7 (1995).

8 The frequency and severity of retinal redetachment in postoperative  
9 patients in all five groups is monitored over a period of nine months using  
10 ophthalmologic examination techniques that will be familiar to those of  
11 ordinary skill in the art. Patients receiving 40 mg oral 13-cis retinoic acid  
12 exhibit significantly reduced incidence of retinal redetachment when  
13 compared with control patients, patients receiving 4 mg oral 13-cis retinoic  
14 acid twice daily or patients receiving 40 mg oral AGN 193109 twice daily.  
15 Examination of the patient group receiving the combination of 40 mg oral  
16 AGN 193109 and 4 mg oral 13-cis retinoic acid twice daily for four weeks  
17 postoperatively demonstrates the therapeutic outcome in this patient group is  
18 equal to or better than those patients receiving 40 mg oral 13-cis retinoic acid  
19 twice daily for four weeks postoperatively. This result demonstrates that the  
20 AGN 193109 negative hormone improves the therapeutic index of an RAR  
21 agonist by virtue of decreasing the frequency and severity of retinal  
22 redetachment in PVR patients.

### 23 Generalized Assay for Identifying Nuclear Receptor Negative Hormones

24 We have demonstrated above that AGN 193109 can function as a  
25 negative hormone capable of repressing the basal transcriptional activity of  
26 RAR nuclear receptors. Further, we have described an assay using CV-1  
27 cells co-transfected with the ERE-tk-Luc-luciferase reporter plasmid and the  
28 ER-RXR- $\alpha$  and RAR- $\gamma$ -VP-16 receptor expression plasmids for  
29 distinguishing RAR ligands that are simple antagonists from those having

1 negative hormone activity.

2 We have concluded that RAR negative hormones mediate repression  
3 of RAR-mediated transcriptional activity by promoting increased interaction  
4 between the RAR and NCPs. Further, we have demonstrated that AGN  
5 193109 can potentiate the effects of agonists of other nuclear receptors in a  
6 manner consistent with the mutual sharing of NCPs between members of the  
7 steroid superfamily of nuclear receptors. As such, ligands can be designed  
8 and screened to identify compounds having negative hormone activity at  
9 these non-RAR nuclear receptors.

10 Our method of RAR negative hormone screening based on the use of  
11 CV-1 cells co-transfected with the ERE-tk-Luc luciferase reporter plasmid  
12 and the ER-RXR- $\alpha$  and RAR- $\gamma$ -VP-16 receptor expression plasmids can be  
13 adapted generally such that the RAR- $\gamma$  moiety of the RAR- $\gamma$ -VP-16 plasmid  
14 is converted to that of peroxisome proliferator-activated receptors (PPAR),  
15 vitamin D receptor (VDR), thyroid hormone receptor (T3R) or any other  
16 steroid superfamily nuclear receptor capable of heterodimerizing with RXR.  
17 CV-1 cells co-transfected with such plasmids would express high basal levels  
18 of luciferase activity. Ligands capable of binding the ligand binding domain  
19 of the receptor substituted for the RAR- $\gamma$  moiety can be easily screened for  
20 negative hormone activity by measuring their ability to repress luciferase  
21 activity.

22 For steroid superfamily nuclear receptors that do not heterodimerize  
23 with RXR (e.g., glucocorticoid and estrogen receptors) the same end result  
24 can be achieved using GR-VP-16 or ER-VP-16 receptors and a luciferase  
25 reporter plasmid consisting of the appropriate glucocorticoid or estrogen  
26 response element fused to a heterologous promoter element and luciferase or  
27 other reporter gene. An essential feature of a generalized negative hormone  
28 screening assay is the inclusion of at least the ligand binding domain of the  
29 particular nuclear receptor for which inverse agonists are to be screened and



1 a method for localizing the nuclear receptor ligand binding domain to the  
2 promoter of a reporter gene. This could be achieved using the receptors's  
3 natural DNA binding site, or alternatively by construction of a chimeric  
4 receptor having a heterologous DNA binding domain and corresponding use  
5 of a reporter gene which is under control of a DNA regulatory element which  
6 is recognized by the heterologous DNA binding domain. In a preferred  
7 embodiment, the plasmid expressing the nuclear receptor for which inverse  
8 agonists are to be screened would express this nuclear receptor as a fusion  
9 protein containing a constitutive activation domain, such as the HSV VP-16  
10 activation domain, in order to provide allow high basal activity. This high  
11 basal activity would effectively increase assay sensitivity, thereby allowing  
12 analysis of nuclear receptor ligands which repress basal transcriptional activity  
13 in the absence of added nuclear receptor agonist.

14 The following Example illustrates one method that can be used to  
15 screen for compounds having negative hormone activity at the thyroid  
16 hormone receptor.

#### 17 Example 26

#### 18 Method of Identifying Thyroid Hormone Receptor

#### 19 Negative Hormones

20 CV-1 cells are co-transfected with the luciferase reporter plasmid  
21 ERE-tk-Luc and the plasmids ER-RXR- $\alpha$  and T3R-VP-16. T3R-VP-16 is  
22 identical to the plasmid RAR- $\gamma$ -VP-16, except the RAR- $\gamma$  moiety of RAR-  
23  $\gamma$ -VP-16 has been substituted by the thyroid hormone receptor cDNA. As  
24 such, T3R-VP-16 expresses a fusion protein containing the activation domain  
25 of HSV VP-16 in frame with the N-terminus of the thyroid hormone  
26 receptor. Standard transfection and cell culture methods are employed for  
27 this purpose. After transfection, cells are rinsed and fed with growth medium  
28 containing 10% fetal calf serum which has been extracted with activated  
29 charcoal. Cells are treated with vehicle alone (ethanol), thyroid hormone (10

1  $10^{-9}$  to  $10^{-10}$  M), or compound TR-1 ( $10^{-9}$  to  $10^{-6}$  M). TR-1 is a synthetic thyroid  
2 hormone receptor ligand which exhibits strong affinity for the thyroid  
3 hormone receptor in competition binding studies, but which does not activate  
4 transfected thyroid hormone receptor in transient cotransfection  
5 transactivation assays using a thyroid hormone responsive reporter gene and a  
6 thyroid hormone receptor expression plasmid. Further, TR-1 is capable of  
7 antagonizing thyroid hormone mediated transactivation and as such is a  
8 thyroid receptor antagonist.

9       Analysis of luciferase activity from CV-1 cell transfected with  
10 ERE-tk-Luc, ER-RXR $\alpha$  and T3R-VP-16 demonstrates a high basal level of  
11 luciferase reporter activity in vehicle-treated cells. Cells treated with thyroid  
12 hormone show a slight increase of luciferase activity in a dose dependent  
13 manner. Cells treated with TR-1 exhibit a dose dependent decrease in  
14 luciferase activity. This indicates that TR-1 exhibits thyroid receptor inverse  
15 agonist activity, presumably due to the increased interaction of a NCP with  
16 the thyroid hormone receptor.

17       The proliferation rate of human primary retinal pigment epithelium  
18 cells is repressed by treatment with RAR agonists. The therapeutic value of  
19 this observation has been demonstrated in post-operative use retinoid therapy  
20 after retinal reattachment surgery. We have above demonstrated the AGN  
21 193109 RAR negative hormone can sensitize primary RPE cells to the  
22 antiproliferative effect of ATRA and 13-*cis* retinoic acid in coadministration  
23 procedures. Further, AGN 193109 was also shown to sensitize RPE cells to  
24 the antiproliferative effects of other nuclear receptor agonists. More  
25 specifically, AGN 193109 sensitized RPE cells to the antiproliferative effects  
26 of the glucocorticoid agonist, dexamethasone, and the thyroid hormone  
27 agonist 3,3',5-triiodothyronine, T<sub>3</sub>. This data was consistent with our working  
28 model wherein AGN 193109 modulated the availability of NCPs that were  
29 shared between the members of the nuclear receptor family. Treatment of

1 RPE cells with the thyroid hormone receptor inverse agonist TR-1 will  
2 similarly alter the availability of shared NCPs such that coadministration with  
3 a non-thyroid receptor agonist, such as the RAR agonist 13-*cis* retinoic acid  
4 will lead to an increased antiproliferative effect upon the RPE cultures as  
5 compared to 13-*cis* retinoic acid as a single agent treatment.

6 The following Example illustrates one method that can be used to  
7 render primary RPE cells more sensitive to the antiproliferative activity of an  
8 RAR agonist. Notably, this Example further illustrates how the activity of  
9 RAR agonists can be potentiated by coadministration with a negative  
10 hormone.

#### 11 Example 27

#### 12 Sensitizing Primary Retinal Pigment Epithelium Cells 13 to the Antiproliferative Effects of RAR Agonists 14 by Coadministration of the TR-1 Thyroid Hormone

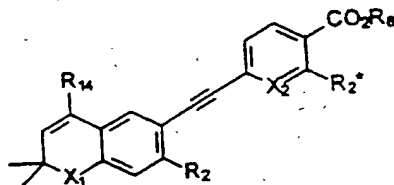
#### 15 Inverse Agonist

16 Human primary RPE cells are obtained and cultured according to  
17 standard methods. The cultured cells are divided into four groups and  
18 treated as follows. Group 1 receives vehicle alone (ethanol). Group 2 is  
19 treated with 13-*cis* retinoic acid at concentrations ranging from  $10^{-11}$  to  $10^{-6}$   
20 M. Group 3 is treated with the thyroid hormone inverse agonist TR-1 at  
21 concentrations ranging from  $10^{-11}$  to  $10^{-1}$  M. Group 4 is co-treated with 13-*cis*  
22 retinoic acid at concentrations ranging from  $10^{-11}$  to  $10^{-6}$  M TR-1. Cells are  
23 refed with fresh growth medium and re-treated with the appropriate  
24 compound every two days for a total of five days of treatment. The  
25 proliferation rate over the duration of the experiment is quantitated via  
26 measurement of the cell number in the cultures using an electric cell counter.  
27 TR-1 treated cells (Group 3) exhibits rates of cellular proliferation  
28 which are essentially the same as control (Group 1) cells and there is no  
29 effect of this inverse agonist upon the measured growth rate of the cultures.

1 Cells treated with 13-*cis* retinoic acid (Group 2) exhibit a dose dependent  
2 decrease in cell number. Comparison of the dose dependent decrease in  
3 cellular proliferation of Group 4 cells (13-*cis* RA and TR-1 coadministration)  
4 with that obtained in Group 3 demonstrates the ability of TR-1 thyroid  
5 hormone receptor inverse agonist coadministration to sensitize RPE cultures  
6 to the antiproliferative effect of 13-*cis* retinoic acid as measured by the shift  
7 in the dose response curve of this RAR agonist to the left in Group 4 as  
8 compared to Group 2 cells.

## WHAT IS CLAIMED IS:

1. A compound of the formula



where  $X_1$  is S or O;

$X_2$  is CH or N;

$R_2$  is H, F,  $CF_3$  or alkoxy of 1 to 6 carbons;

$R_2^*$  H, F, or  $CF_3$ ;

$R_8$  is H, or lower alkyl of 1 to 6 carbons;

$R_{14}$  is unsubstituted phenyl, thienyl or pyridyl, or phenyl, thienyl or pyridyl substituted with one to three  $R_{15}$  groups, where  $R_{15}$  is lower alkyl of 1 to 6 carbons, chlorine,  $CF_3$ , or alkoxy of 1 to 6 carbons, or a pharmaceutically acceptable salt of said compound.

2. A compound in accordance with Claim 1 where  $X_1$  is S.

3. A compound in accordance with Claim 2 where  $X_2$  is CH.

4. A compound in accordance with Claim 2 where  $R_{14}$  is phenyl, 2-thienyl, or 3-pyridyl unsubstituted or substituted with one lower alkyl group having 1 to 6 carbons.

5. A compound in accordance with Claim 2 where  $R_2$  is H or F.

6. A compound in accordance with Claim 2 where  $R_2^*$  is H or F.

7. A compound in accordance with Claim 2 where  $X_2$  is N.

8. A compound in accordance with Claim 1 where  $X_1$  is O.

9. A compound in accordance with Claim 8 where  $X_2$  is CH.

10. A compound in accordance with Claim 8 where  $R_{14}$  is phenyl, 2-

1 thienyl, or 3-pyridyl unsubstituted or substituted with one lower alkyl group  
2 having 1 to 6 carbons.

3 11. A compound in accordance with Claim 8 where  $R_2$  is H or F.

4 12. A compound in accordance with Claim 8 where  $R_2^*$  is H or F.

5 13. A method of treating a pathological condition in a mammal, said  
6 condition associated with a retinoic acid receptor activity, said method  
7 comprising administering to said mammal a retinoid antagonist or negative  
8 hormone capable of binding to a retinoic acid receptor subtype selected from  
9 the group consisting of  $RAR_\alpha$ ,  $RAR_\beta$  and  $RAR_\gamma$ , said antagonist or negative  
10 hormone being administered in an amount pharmaceutically effective to  
11 provide a therapeutic benefit against said pathological condition in said  
12 mammal and wherein said antagonist or negative hormone is a compound in  
13 accordance with Claim 1.

14 14. The method of Claim 13 wherein the pathological condition is  
15 the toxicity or undesired side effects resulting from administration of a  
16 retinoid compound to said mammal, and wherein said therapeutic benefit is  
17 the prevention or amelioration of said toxicity or undesired side effects.

18 15. The method of Claim 13 wherein the retinoid antagonist or  
19 negative hormone is administered in order to cure or ameliorate a pre-  
20 existing pathological condition caused by intake of a retinoid drug or vitamin  
21 A or vitamin A precursors by the mammal.

22 16. The method of Claim 13 wherein the retinoid antagonist or  
23 negative hormone is administered topically to block or ameliorate undesired  
24 topical side effects of a retinoid drug administered for a therapeutic purpose.

25 17. The method of Claim 13 wherein the retinoid antagonist or  
26 negative hormone is administered topically to block or ameliorate undesired  
27 topical side effects of a retinoid drug administered systemically for a  
28 therapeutic purpose.

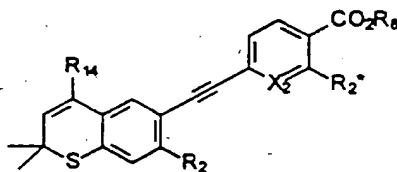
29 18. The method of Claim 13 wherein the retinoid antagonist or

1 negative hormone is administered topically to treat a pre-existing condition or  
2 side effect caused by a retinoid drug or vitamin A.

3 19. The method of Claim 13 wherein the retinoid antagonist or  
4 negative hormone is administered systemically to treat a pre-existing  
5 condition or side effect caused by a retinoid drug or vitamin A.

6 20. The method of Claim 13 wherein the retinoid antagonist or  
7 negative hormone is administered systemically to block or ameliorate bone  
8 toxicity caused by coadministration of a retinoid drug or vitamin A.

9 21. A compound of the formula



16 wherein  $X_2$  is CH or N;

17  $R_2$  is H, F or  $OCH_3$ ;

18  $R_2^*$  is H or F;

19  $R_8$  is H, or lower alkyl of 1 to 6 carbons, and

20  $R_{14}$  is selected from the group consisting of phenyl, 4-(lower-  
21 alkyl)phenyl, 5-(lower alkyl)-2-thienyl, and 6-(lower alkyl)-3-pyridyl where  
22 lower alkyl has 1 to 6 carbons, or a pharmaceutically acceptable salt of said  
23 compound.

24 22. A compound in accordance with Claim 21 where  $X_2$  is CH.

25 23. A compound in accordance with Claim 22 where  $R_2$  is H.

26 24. A compound in accordance with Claim 23 where  $R_2^*$  is H.

27 25. A compound in accordance with Claim 24 where the  $R_{14}$  group  
28 is selected from phenyl, 4-methylphenyl, 4-ethylphenyl, 4-*i*-propylphenyl, 4-*i*-  
29 butylphenyl, 5-methyl-2-thienyl, 5-ethyl-2-thienyl, 5-*i*-butyl-2-thienyl and 6-

1 methyl-3-pyridyl.

2 26. A compound in accordance with Claim 25 where  $R_8$  is ethyl or  
3 H, or a pharmaceutically acceptable salt of said compound.

4 27. A compound in accordance with Claim 23 where  $R_2^*$  is F.

5 28. A compound in accordance with Claim 27 where the  $R_{14}$  group  
6 is selected from 4-methylphenyl and 4-ethylphenyl.

7 29. A compound in accordance with Claim 28 where  $R_8$  is ethyl or  
8 H, or a pharmaceutically acceptable salt of said compound.

9 30. A compound in accordance with Claim 22 where  $R_2$  is F and  $R_2^*$   
10 is H.

11 31. A compound in accordance with Claim 30 where the  $R_{14}$  group  
12 is selected from 4-methylphenyl and 5-methyl-2-thienyl.

13 32. A compound in accordance with Claim 30 where  $R_8$  is ethyl or  
14 H, or a pharmaceutically acceptable salt of said compound.

15 33. A compound in accordance with Claim 22 where  $R_2$  is  $OCH_3$ ,  
16  $R_2^*$  is H and  $R_{14}$  is 4-methylphenyl.

17 34. A compound in accordance with Claim 33 where  $R_8$  is ethyl or  
18 H, or a pharmaceutically acceptable salt of said compound.

19 35. A compound in accordance with Claim 21 where  $X_2$  is N,  
20  $R_2$  is H,  $R_2^*$  is H, and  $R_{14}$  is selected from the group consisting of 4-  
21 methylphenyl and 4-ethylphenyl.

22 36. A compound in accordance with Claim 35 where  $R_8$  is ethyl or  
23 H, or a pharmaceutically acceptable salt of said compound.

24 37. A compound of the formula

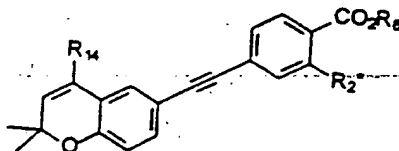
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1 where  $R_2^*$  is H or F;

2  $R_8$  is H, or lower alkyl of 1 to 6 carbons, and

3  $R_{14}$  is selected from the group consisting of phenyl, and 4-(lower-  
4 alkyl)phenyl, where lower alkyl has 1 to 6 carbons, or a pharmaceutically  
5 acceptable salt of said compound.

6 38. A compound in accordance with Claim 37 where  $R_2^*$  is H.

7 39. A compound in accordance with Claim 38 where the  $R_{14}$  group  
8 is selected from phenyl, 4-methylphenyl, 4-ethylphenyl, 4-*i*-propylphenyl, and  
9 4-*t*butylphenyl.

10 40. A compound in accordance with Claim 39 where  $R_8$  is ethyl or  
11 H, or a pharmaceutically acceptable salt of said compound.

12 41. A compound in accordance with Claim 37 where  $R_2^*$  is F.

13 42. A compound in accordance with with Claim 41 where  $R_{14}$  is  
14 selected from the group consisting of 4-methylphenyl and 4-ethylphenyl.

15 43. A compound in accordance with Claim 42 where  $R_8$  is ethyl or  
16 H, or a pharmaceutically acceptable salt of said compound.

17 44. A compound of the formula

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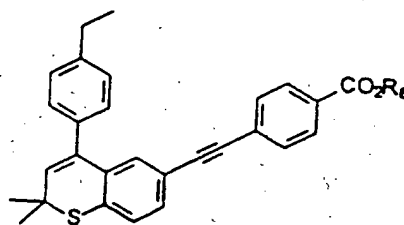
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24



25 where  $R_8$  is H, lower alkyl of 1 to 6 carbons, or a pharmaceutically  
26 acceptable salt of said compound.

27 45. A compound in accordance with Claim 44 where  $R_8$  is H, or a  
28 pharmaceutically acceptable salt of said compound.

29 46. A compound in accordance with Claim 44 where  $R_8$  is ethyl.

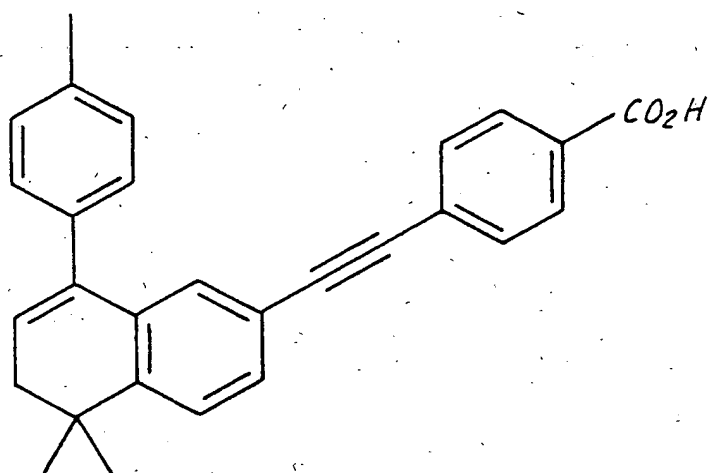
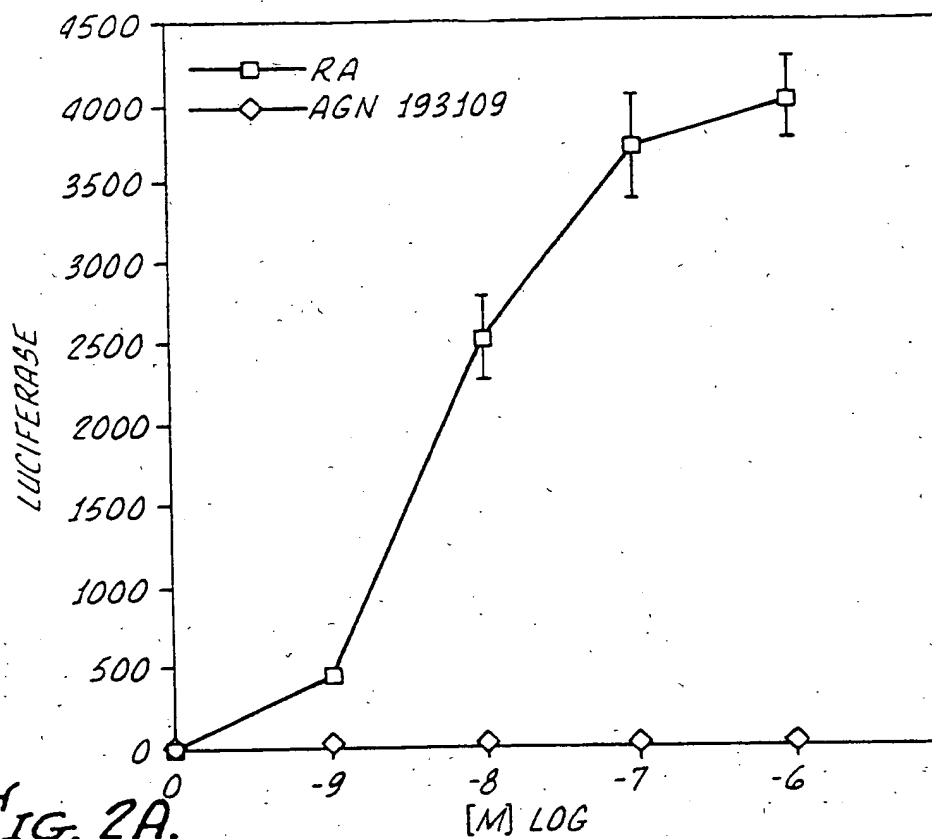
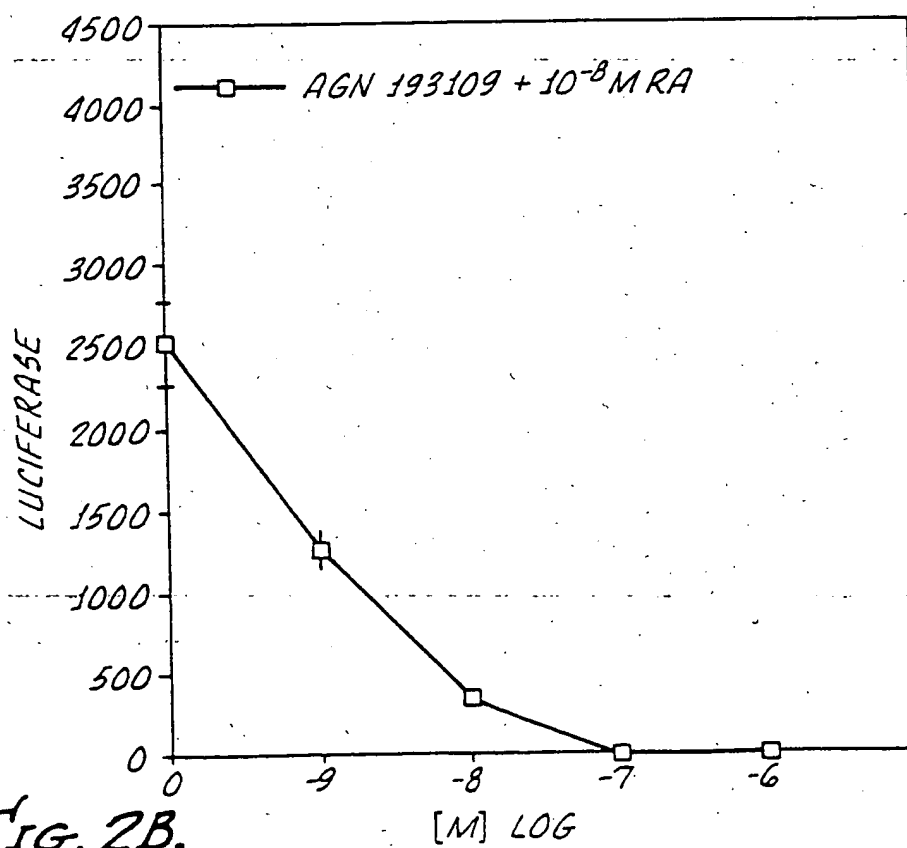


FIG. 1.

FIG. 2A.FIG. 2B.

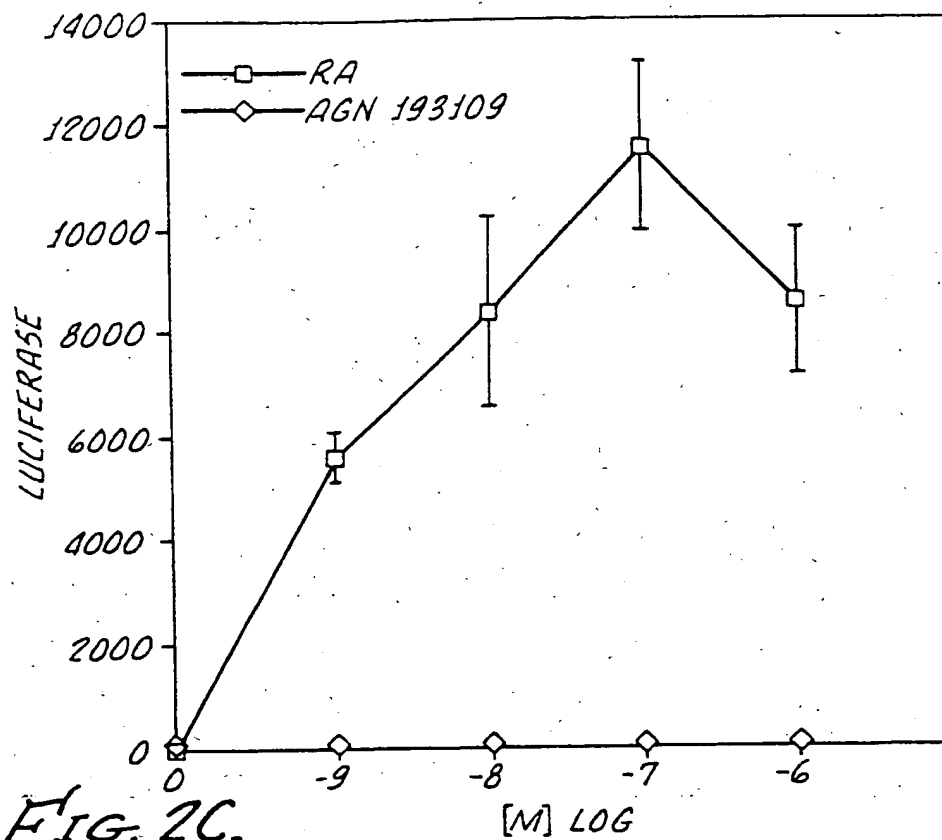


FIG. 2C.

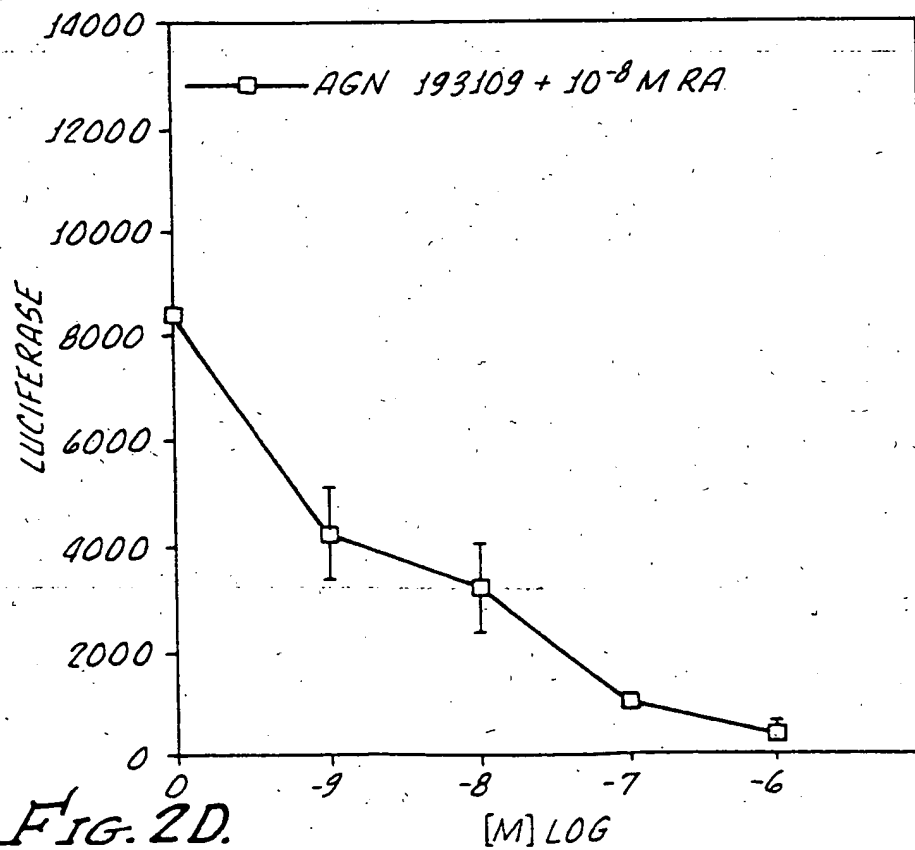
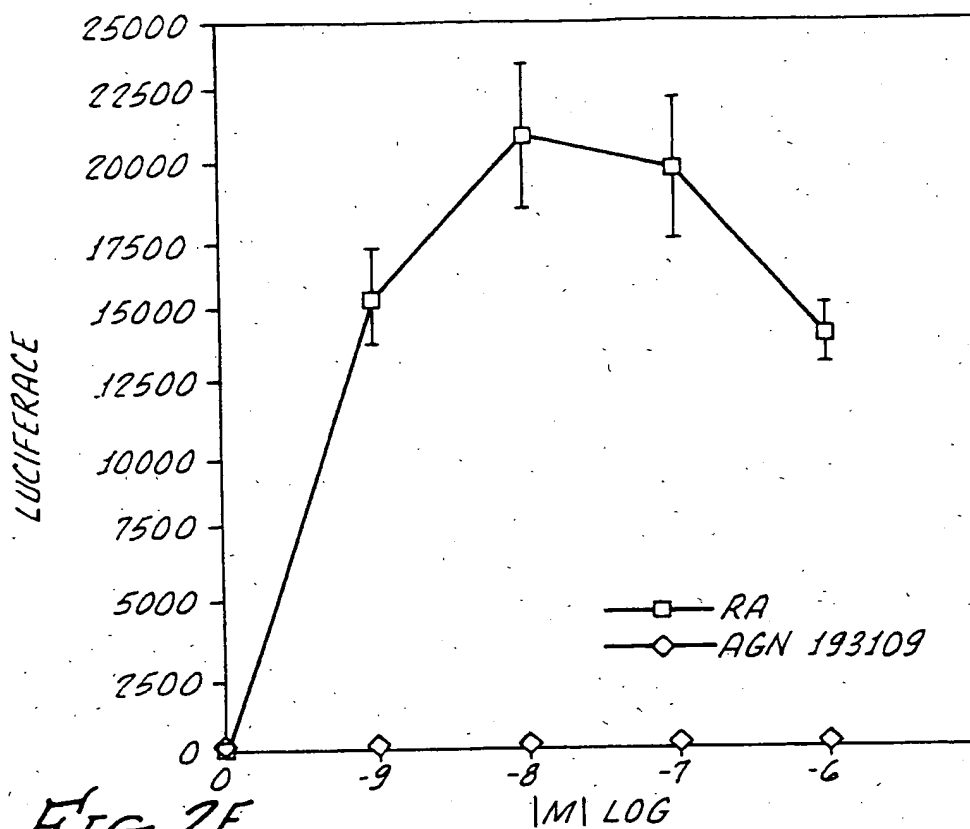
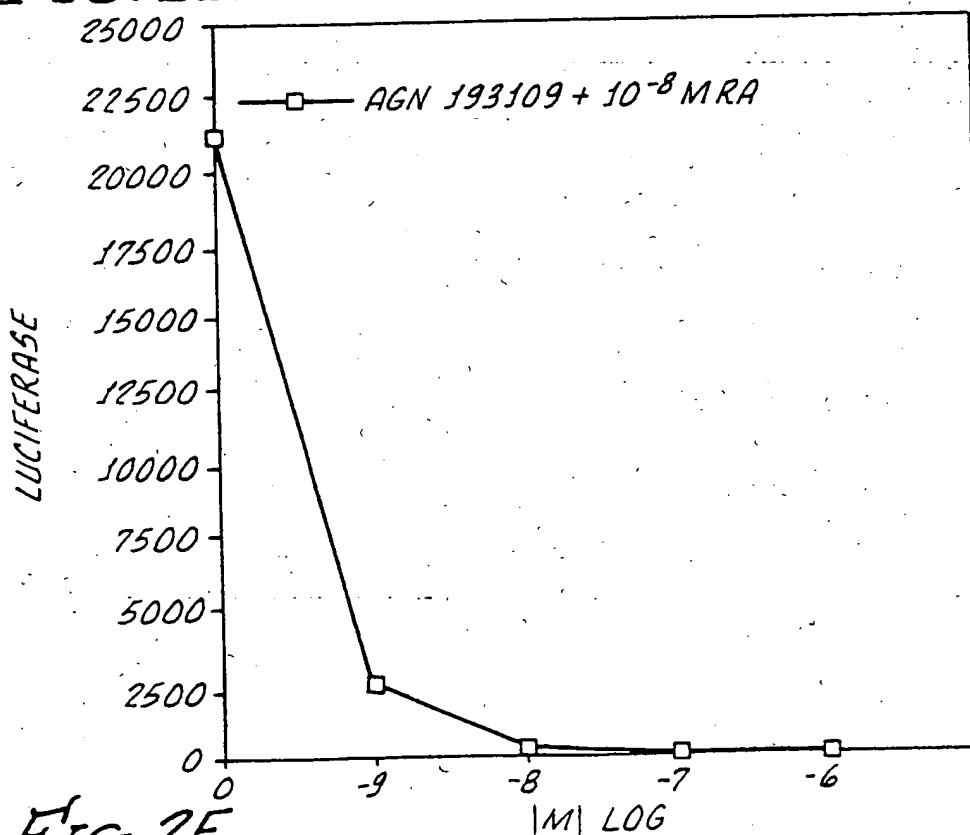


FIG. 2D.

FIG. 2E.FIG. 2F.

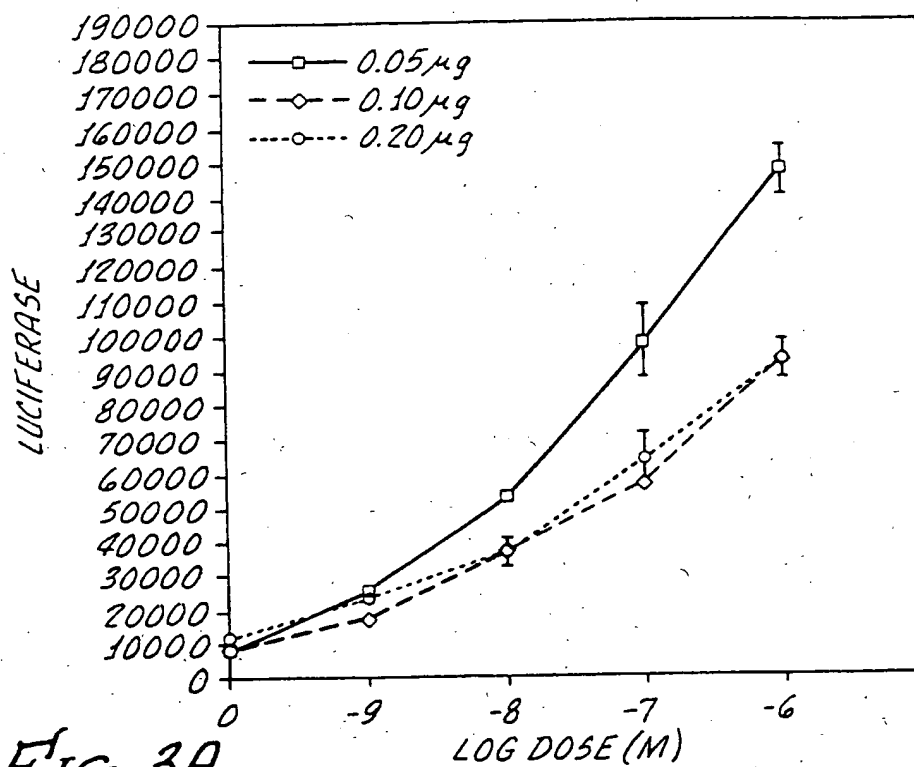


Fig. 3A.

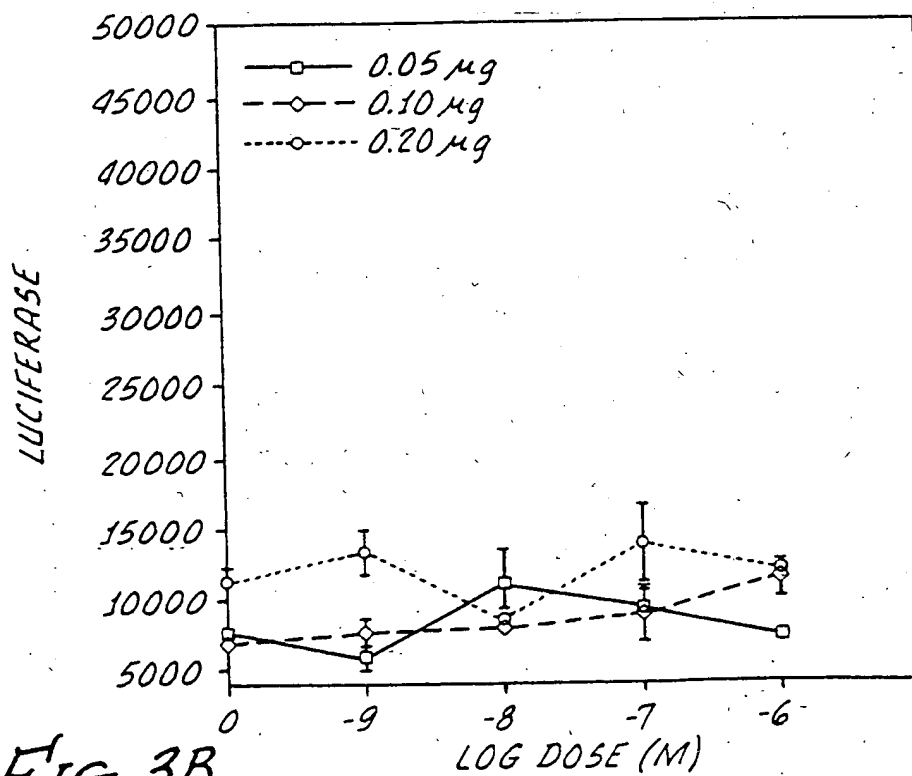
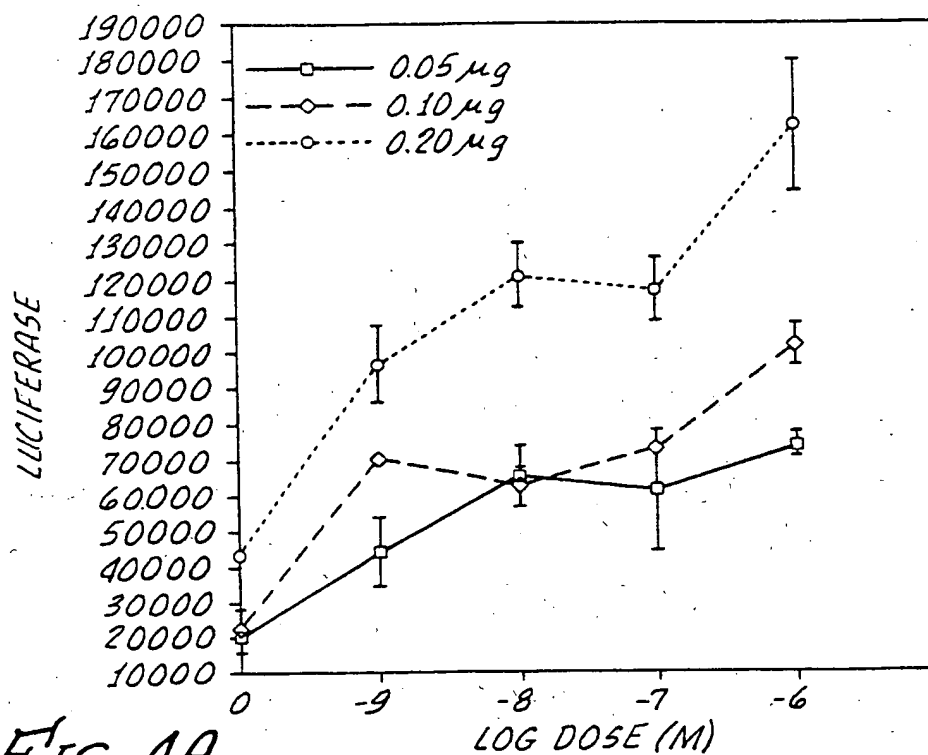
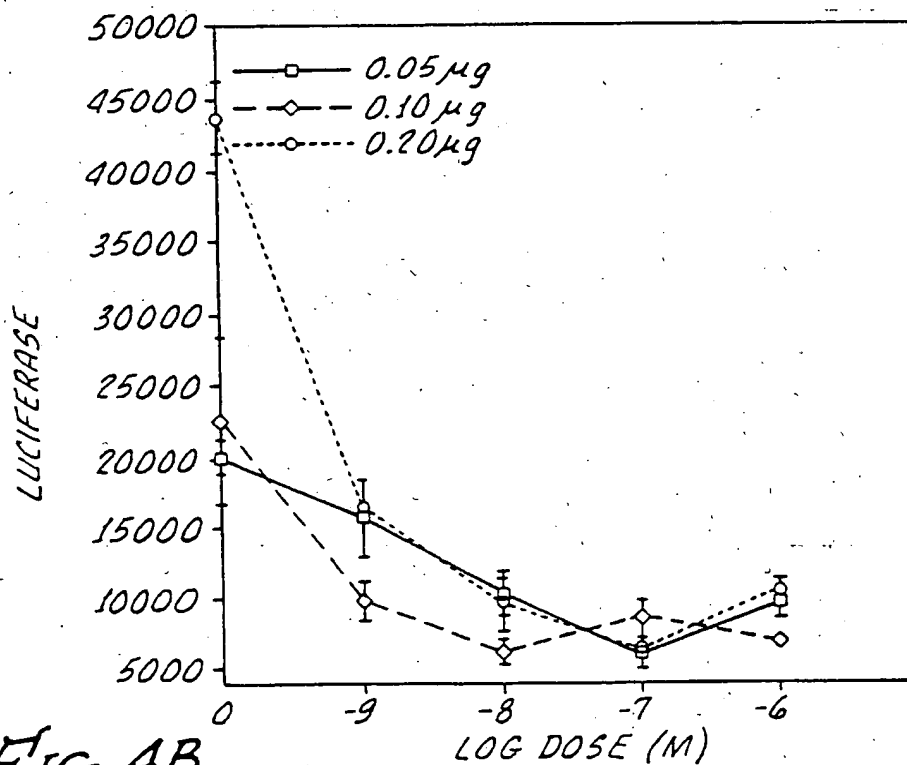


Fig. 3B.

FIG. 4A.FIG. 4B.

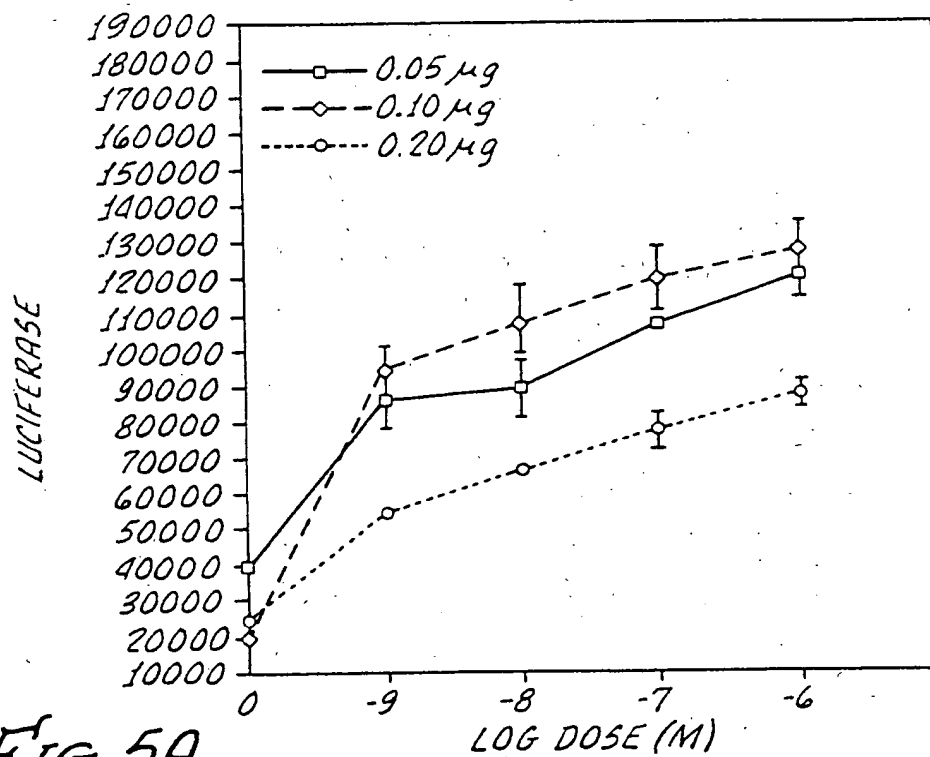


FIG. 5A.

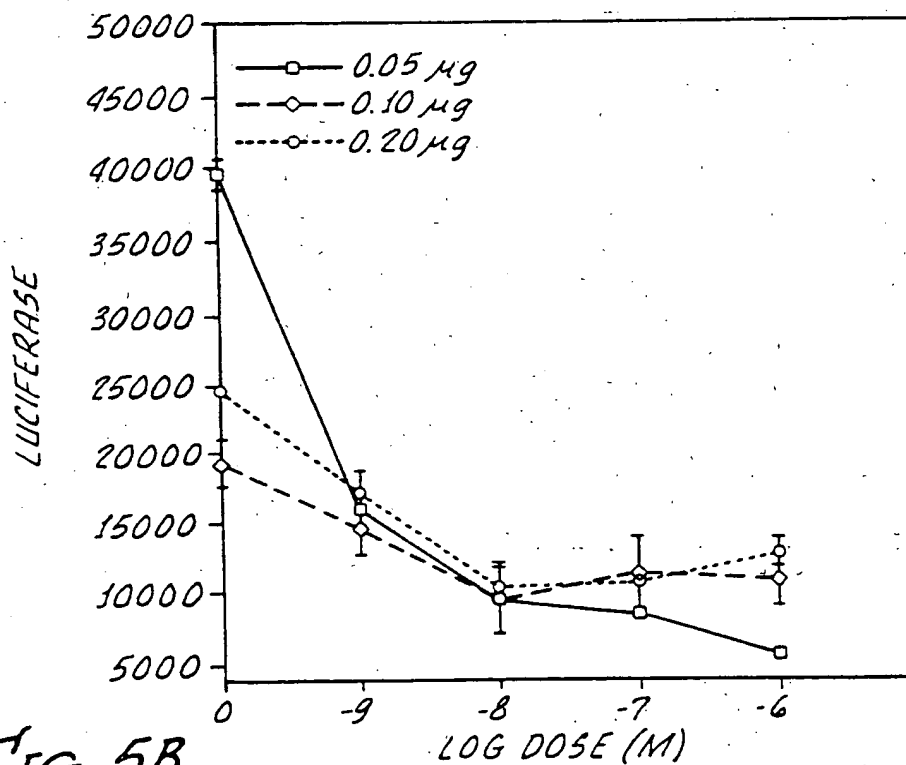


FIG. 5B.



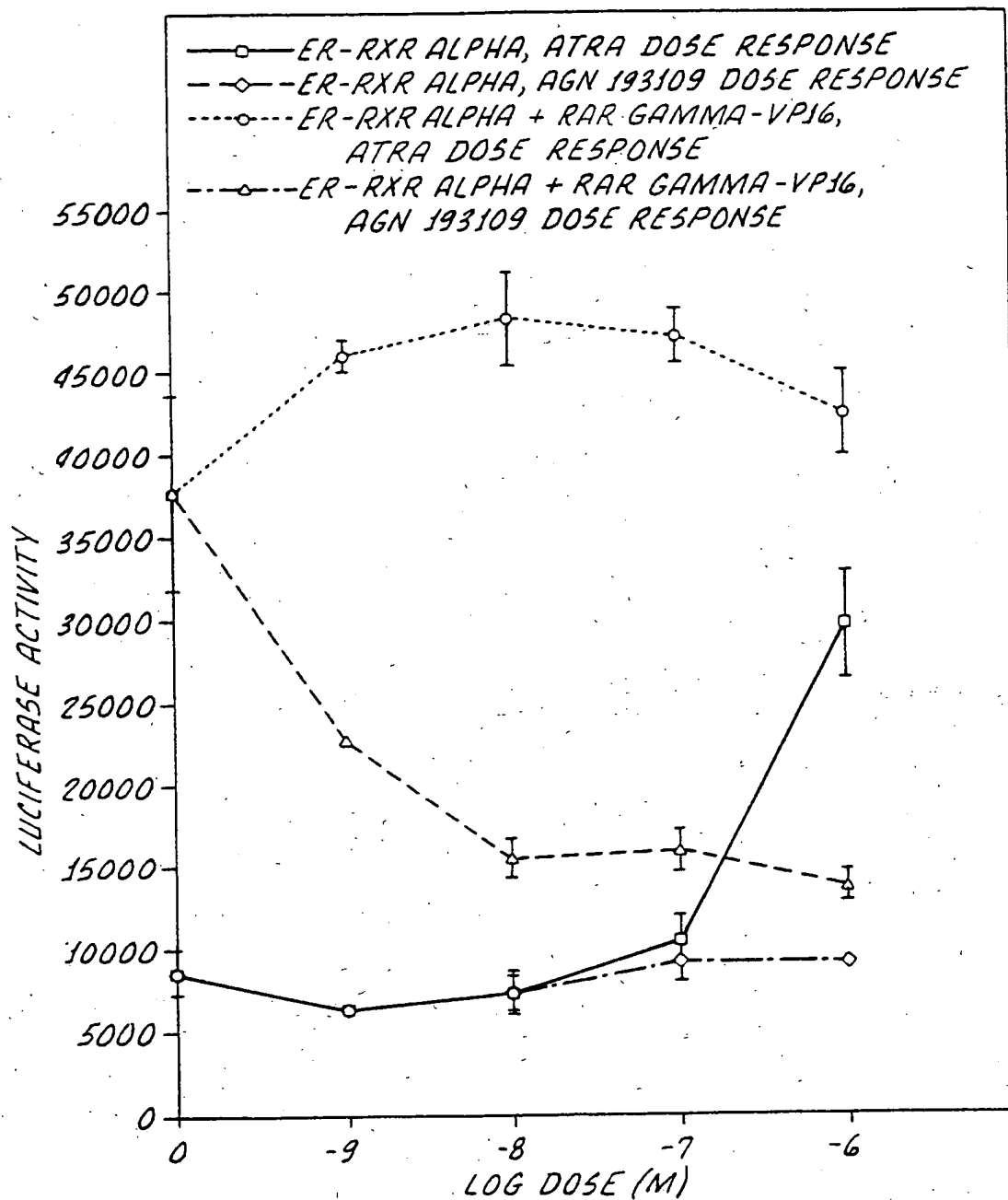
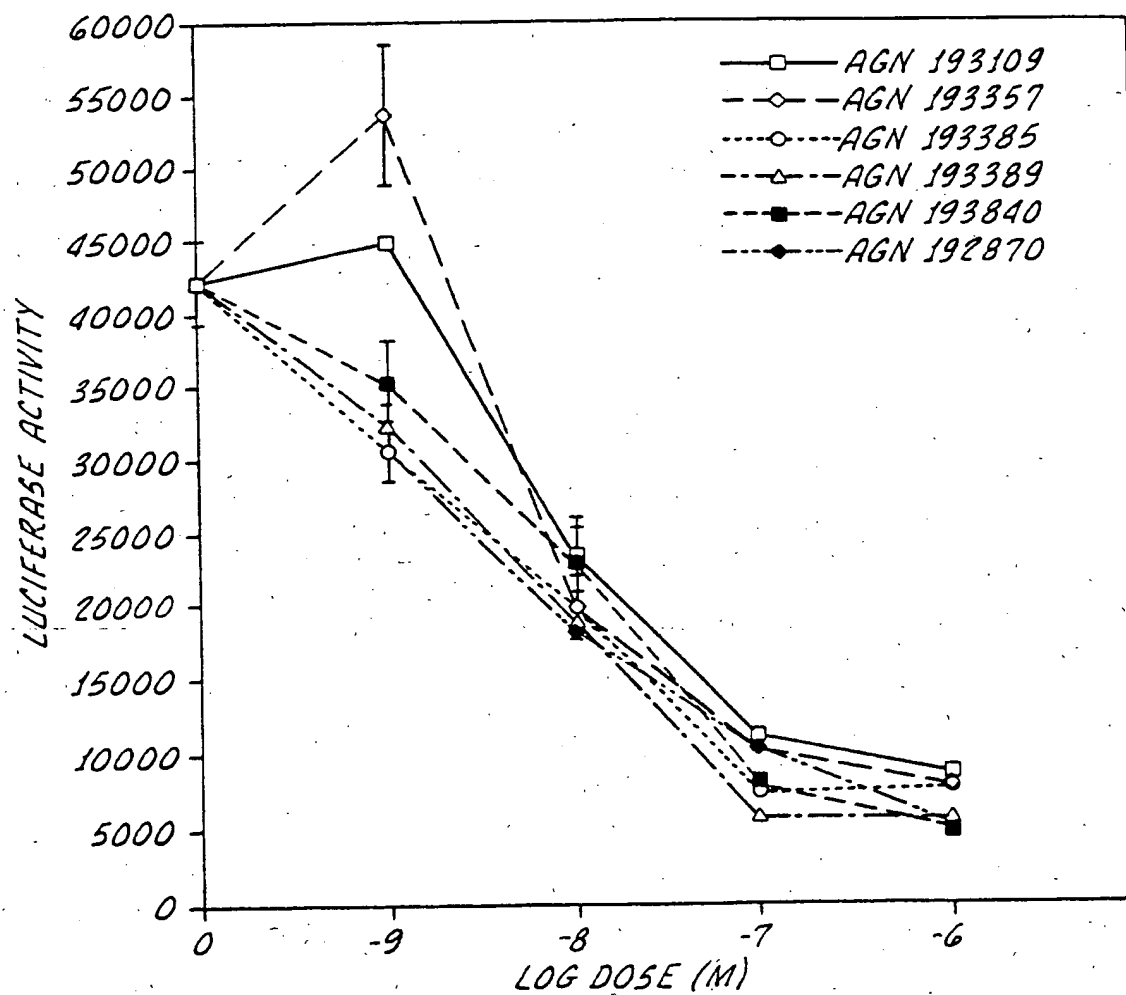
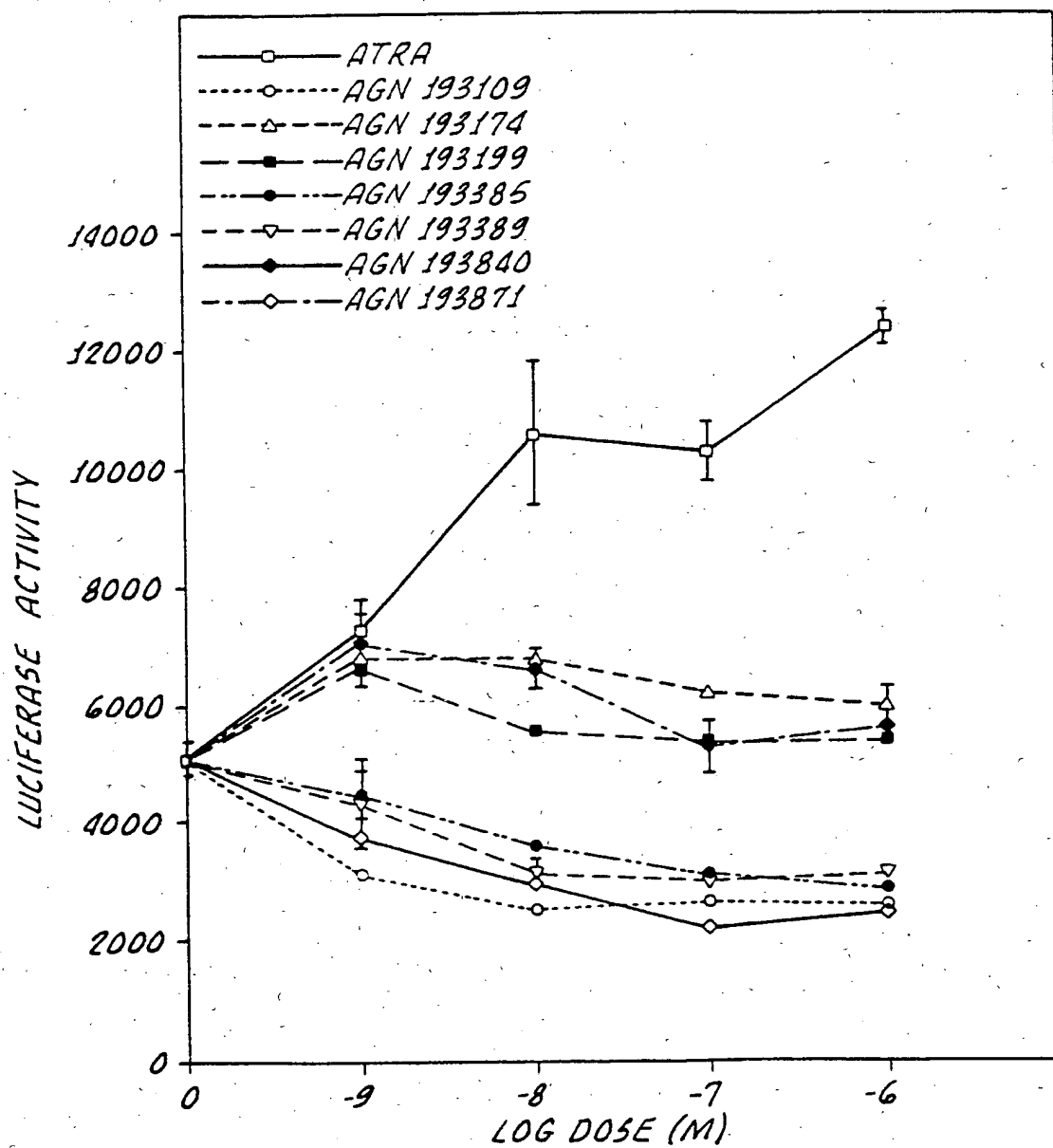
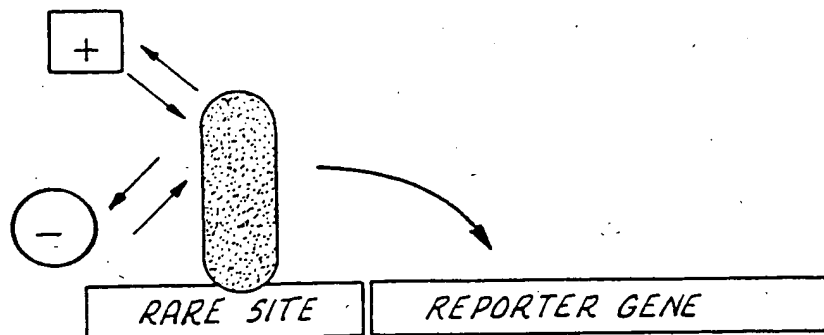
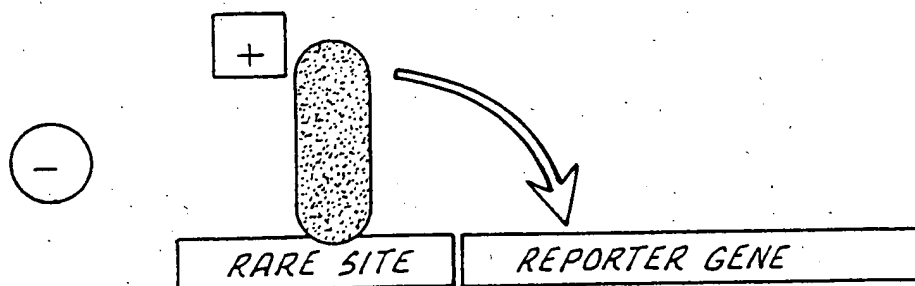
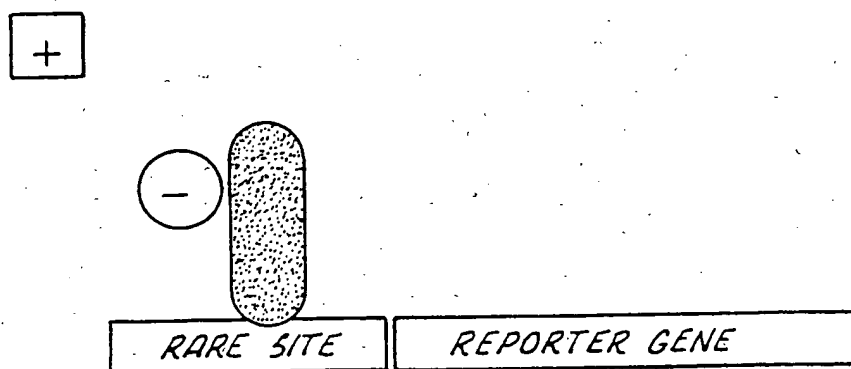


FIG. 6.

FIG. 7.

FIG. 8.

FIG. 9A.FIG. 9B.FIG. 9C.

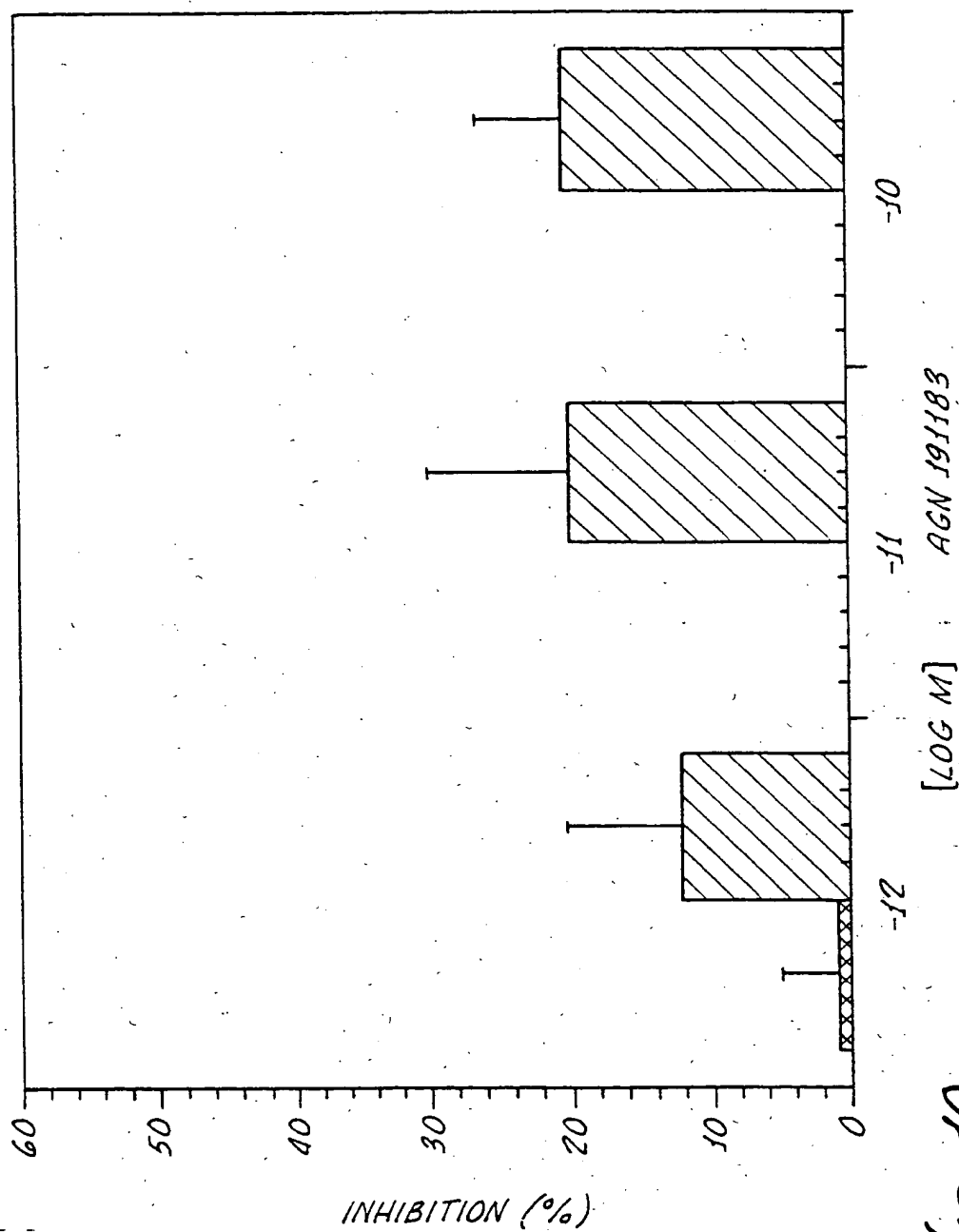


Fig. 10.

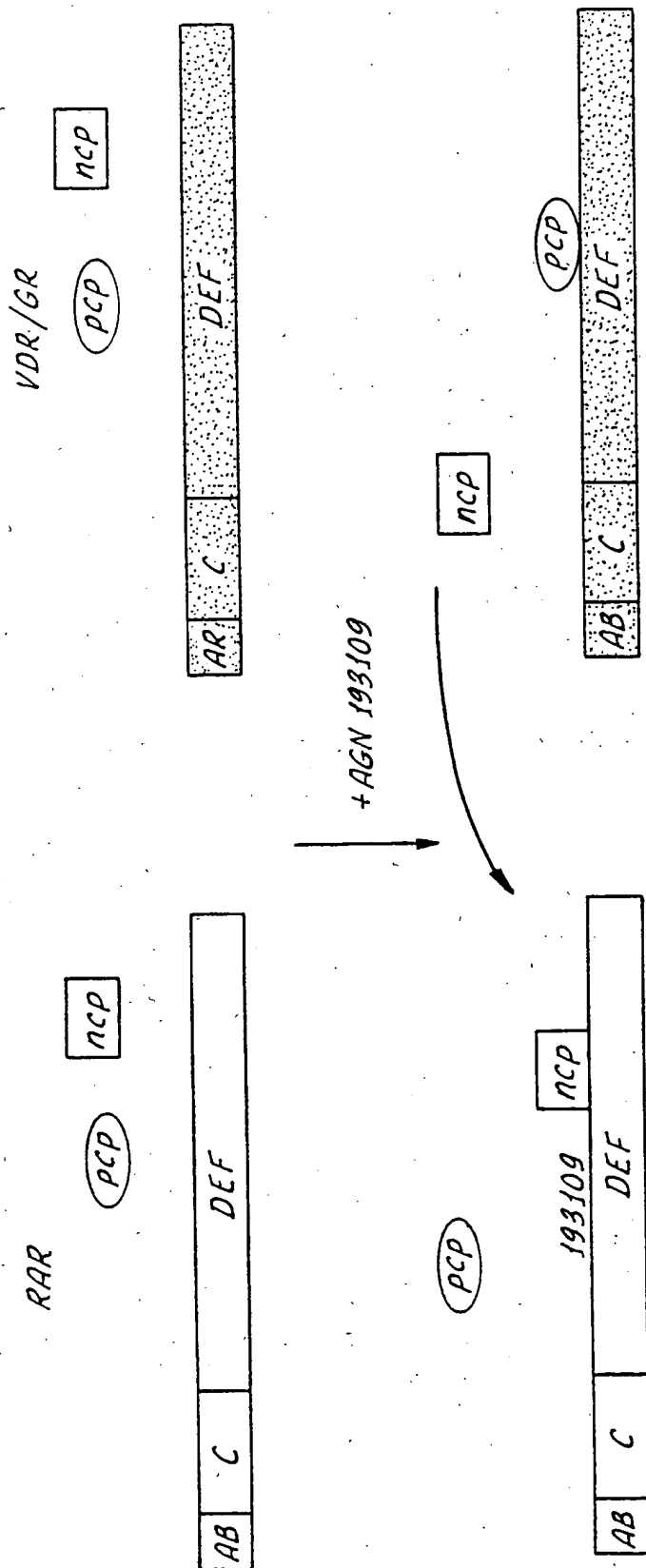


FIG. 11.

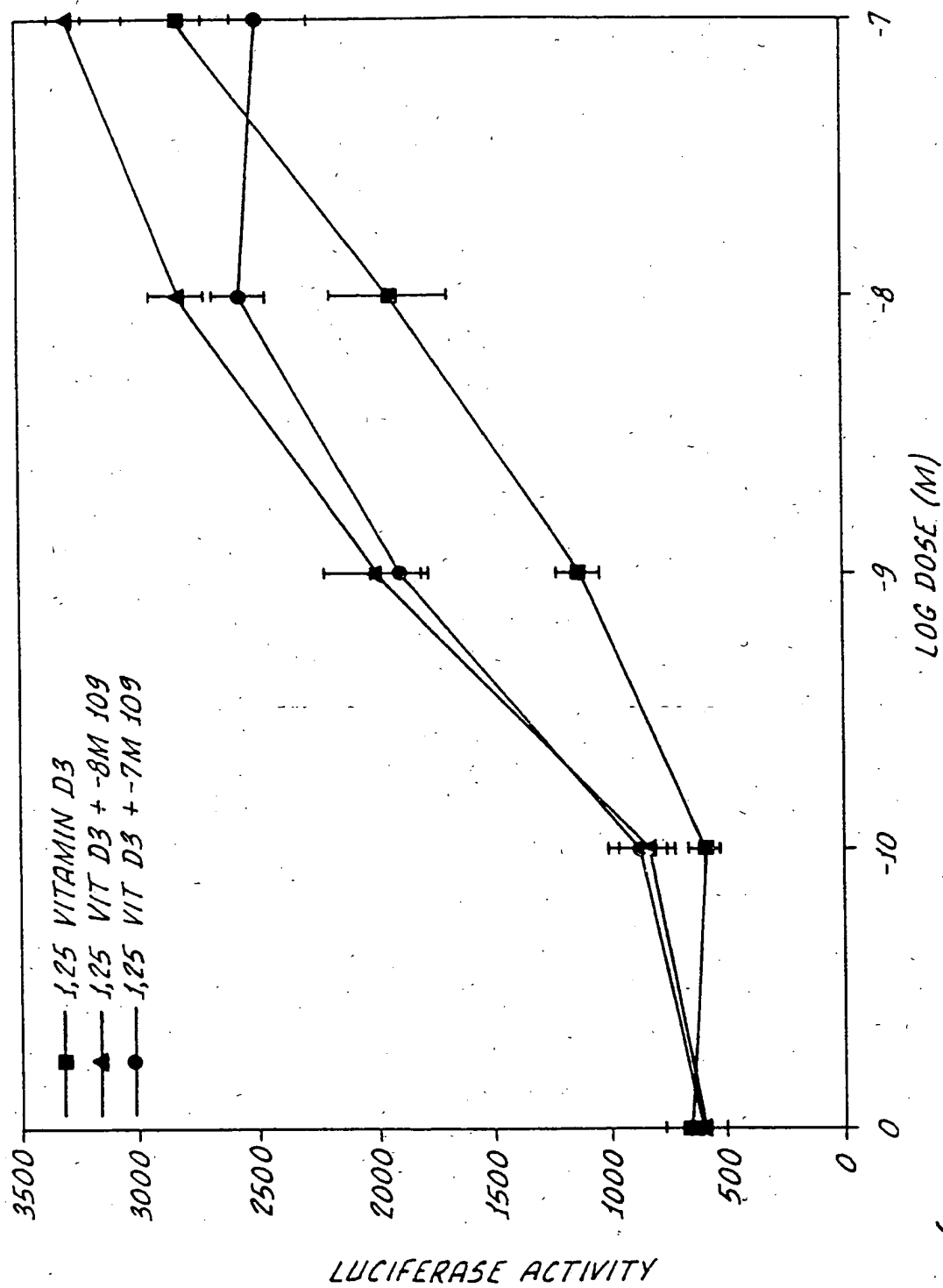


FIG. 12.

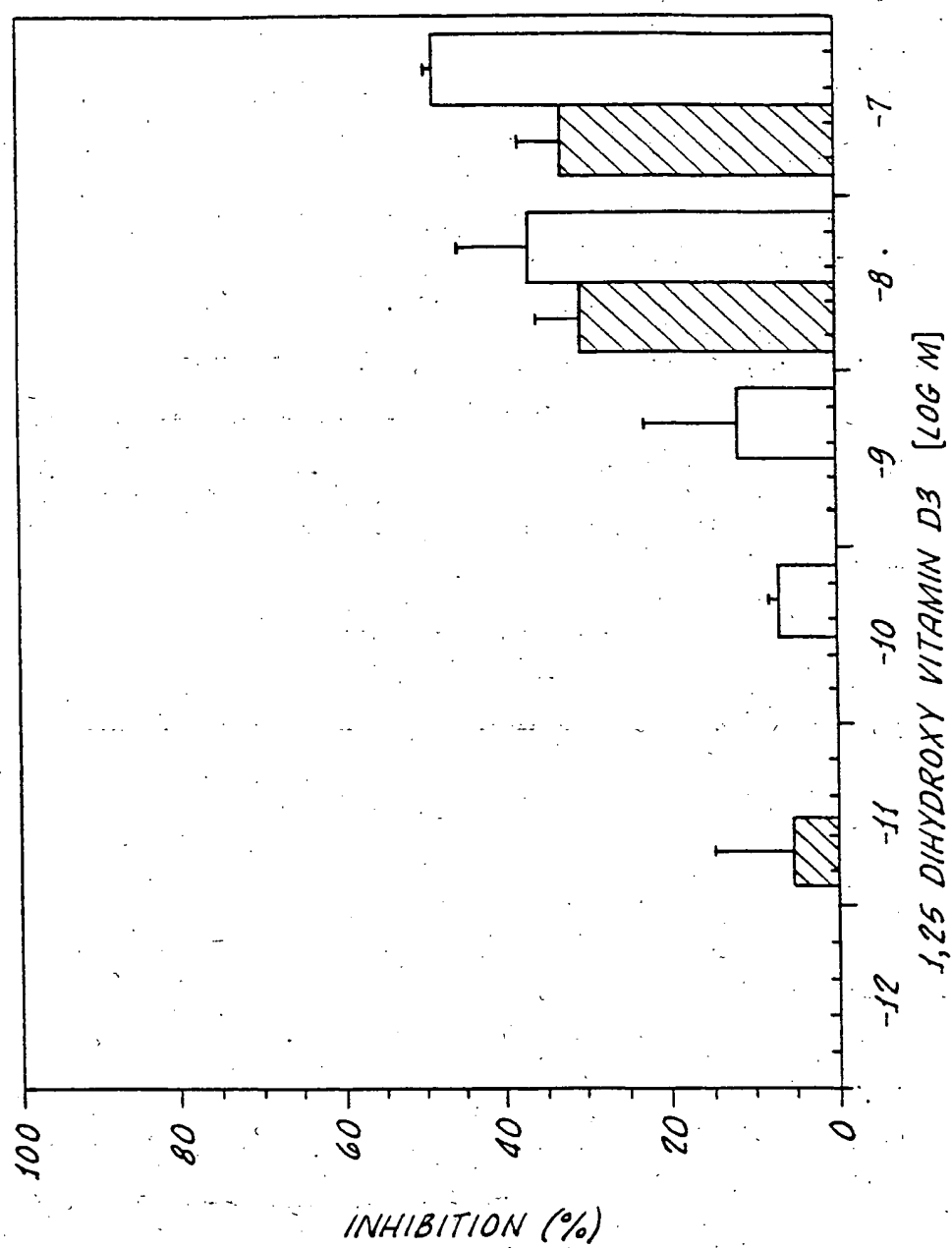
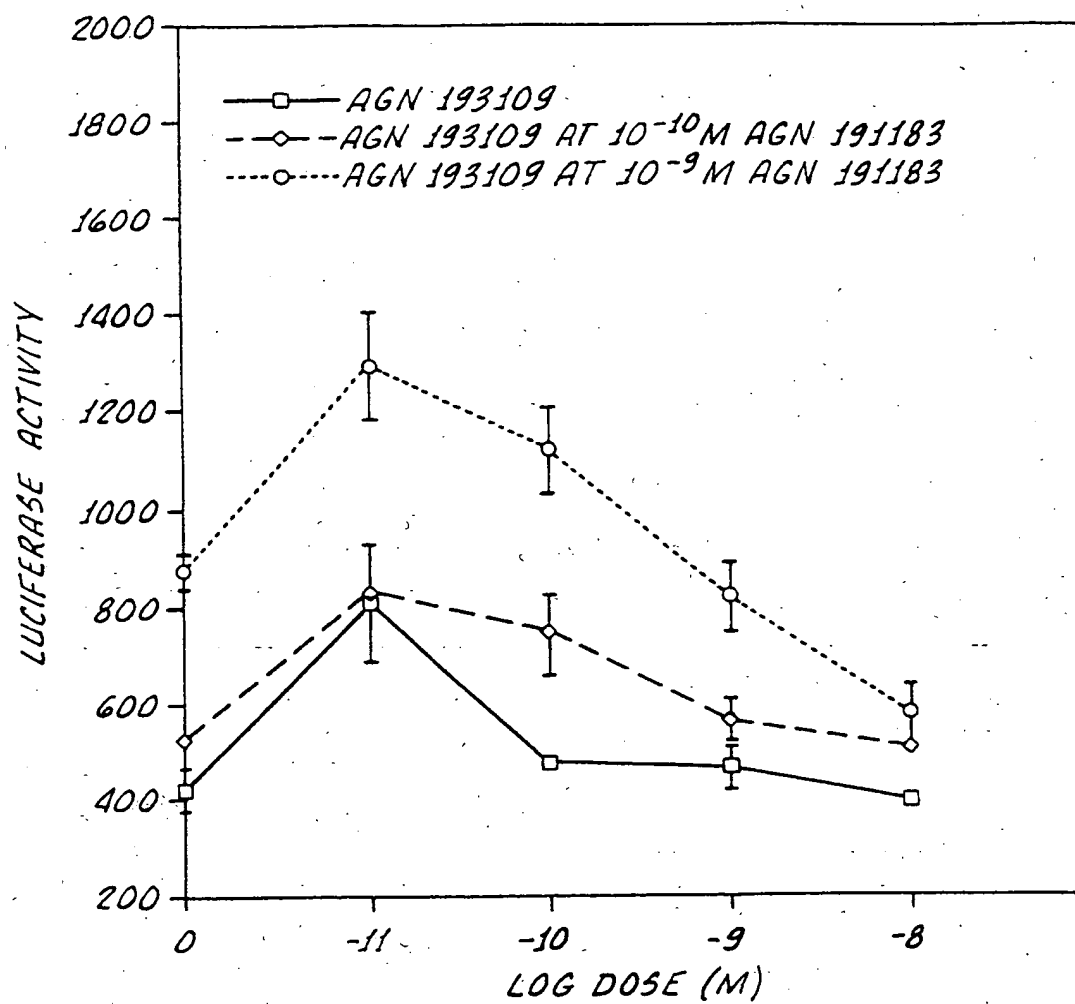
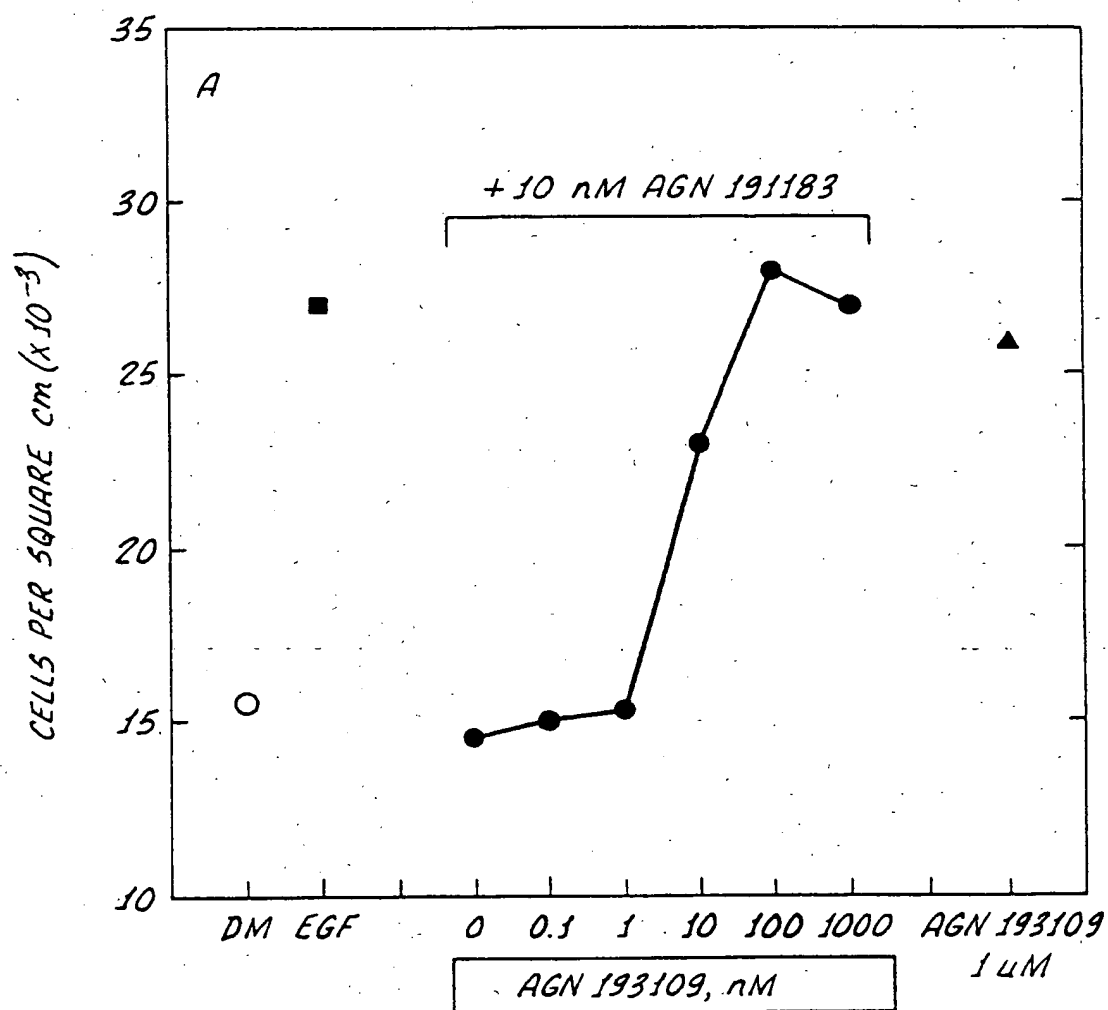


FIG. 13.



*FIG. 14.*

FIG. 15.

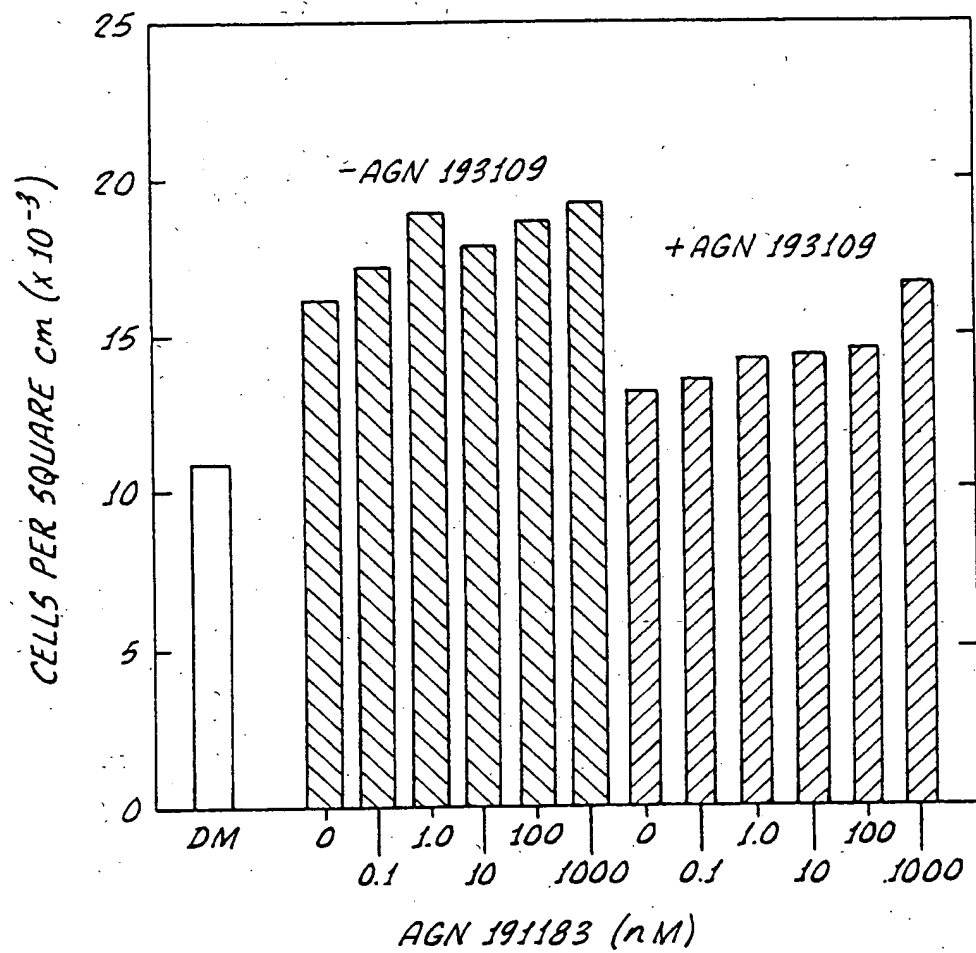
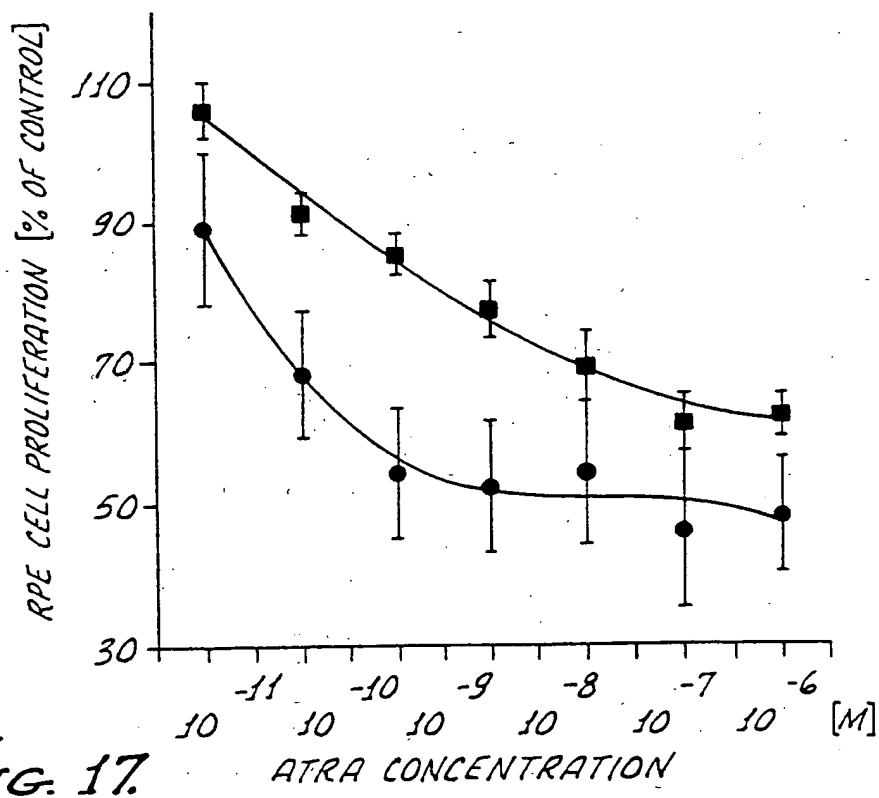
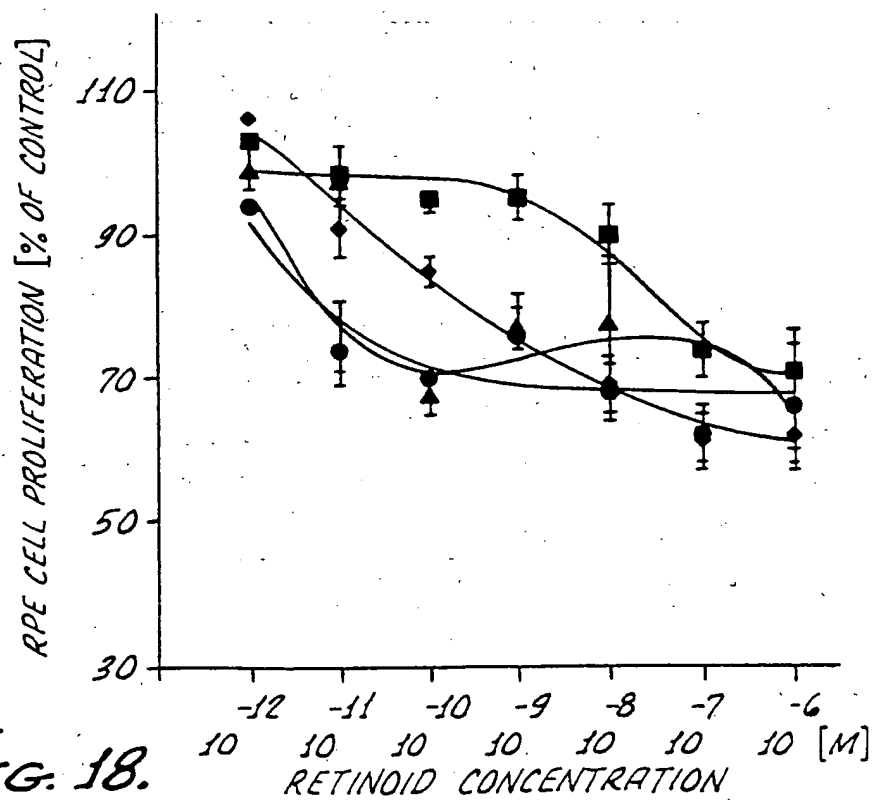
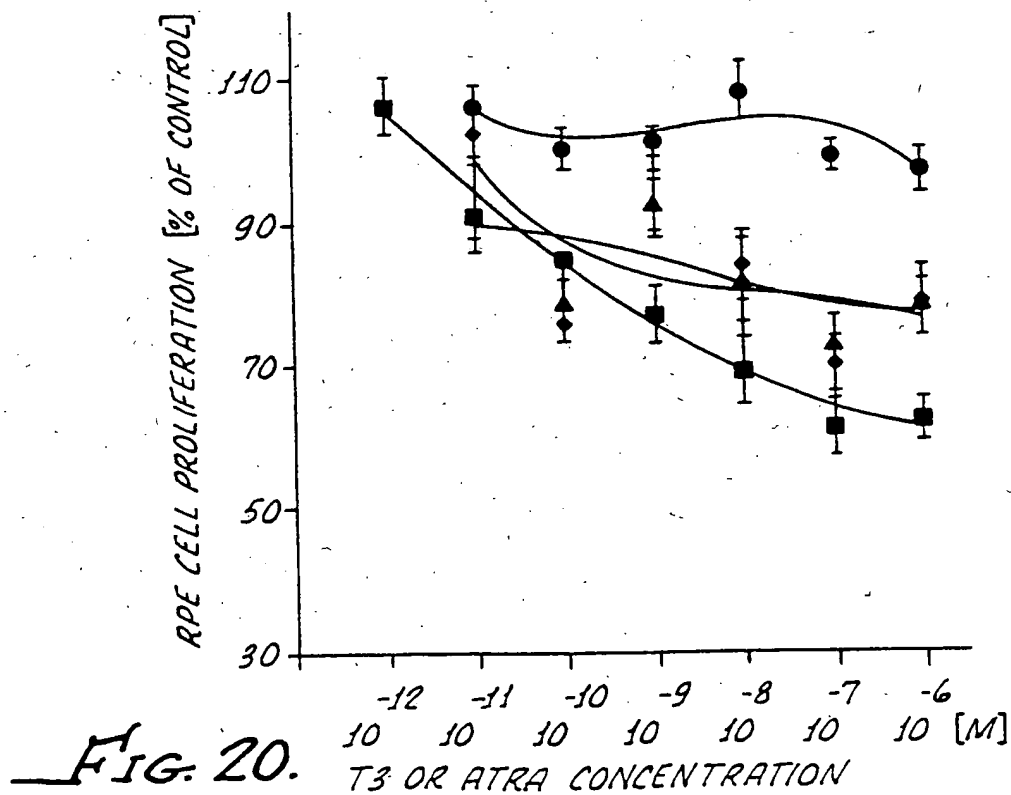
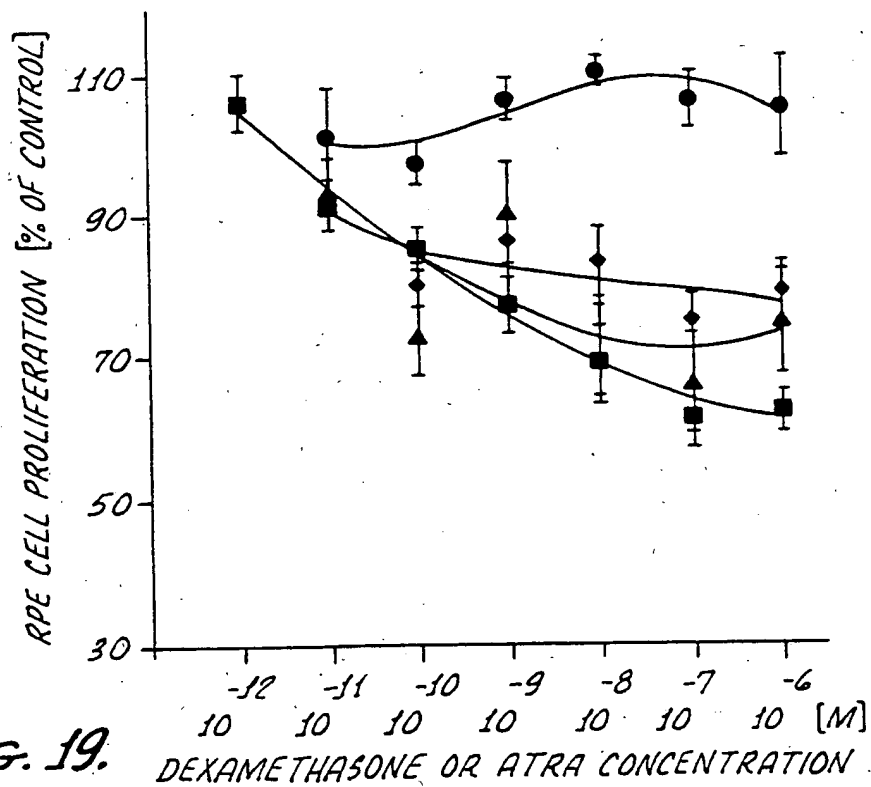


FIG. 16.

FIG. 17.FIG. 18.



# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 98/27314

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07D335/06 C07D311/58 C07D213/55 C07D277/30 C07D333/24  
C07D307/54 C07D409/04 C07D409/06 C07C69/78 C07C233/65  
C07C327/48 A61K31/44 A61K31/335 A61K31/38 A61K31/425

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07D C07C A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 09297 A (ALLERGAN INC ; BEARD RICHARD L (US); DUONG TIEN T (US); JOHNSON ALA) 13 March 1997 see the whole document ---	1-46
A	EP 0 284 288 A (ALLERGAN INC) 28 September 1988 see claims 1,11; examples & US 5 264 578 A cited in the application & US 5 348 972 A cited in the application & US 5 380 877 A cited in the application & US 5 234 926 A cited in the application -----	1,13,21, 37,44



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

### \* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"Z" document member of the same patent family

Date of the actual completion of the international search

6 May 1999

Date of mailing of the international search report

20/05/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Bosma, P.

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 98/27314

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 13-20  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claims 13-20  
are directed to a method of treatment of the human/animal  
body, the search has been carried out and based on the alleged  
effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such  
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all  
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment  
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report  
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is  
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/27314

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